This is provisional English translation of an excerpt from the original full report.

Risk Assessment Report
Deoxynivalenol and Nivalenol (Mycotoxin)

Food Safety Commission of Japan (FSCJ)
November 2010

Contents

Chronology of Discussions

19	March	2009	The 278th Meeting of the Food Safety Commission (decided to conduct as self-tasking risk assessments)
1	May	2009	The 12th Meeting of the Expert Panel on Mycotoxin and Natural Toxins (EPMNT)
17	September	2009	The 13th Meeting of the EPMNT
4	December	2009	The 14th Meeting of the EPMNT
5	February	2010	The 15th Meeting of the EPMNT
15	March	2010	The 16th Meeting of the EPMNT
18	June	2010	The 17th Meeting of the EPMNT
16	September	2010	The 348th Meeting of the Food Safety Commission (Report)
17	September	2010	Call for public opinions and information (through October 16)
26	October	2010	The 19th Meeting of the EPMNT
16	November	2010	Report from the Chairperson of the EPMNT to the Chairperson of the Food Safety Commission
18	November	2010	The 356th Meeting of the Food Safety Commission (Report) (Notice to the Minister of Health, Labour and Welfare and the Minister of Agriculture, Forestry and Fisheries dated the same date)

List of members of the Food Safety Commission Japan (FSCJ)

Up to 30 June 2009
Takeshi Mikami, Chairperson of FSCJ
Naoko Koizumi, Deputy Chairperson
Taku Nagao
Masao Hirose
Kazumasa Nomura
Keiko Hatae
Seiichi Honma

From 1 July 2009
Naoko Koizumi, Chairperson of FSCJ
Takeshi Mikami, Deputy Chairperson
Taku Nagao
Masao Hirose
Kazumasa Nomura
Keiko Hatae
Masatsune Murata

List of members of the EPMNT, the Food Safety Commission Japan (FSCJ)

Up to September 30, 2009
Motoyoshi Satake, Chairperson
Kosuke Takatori, Deputy Chairperson
Osamu Arakawa
Yasukatsu Oshima
Ken-ichi Kawai
Susumu Kumagai
Yukihiro Goda
Yoshiko Konishi
Kazuo Shiomi
Makoto Shibuya
Masatake Toyoda
Nobuhiro Fusetani
Kimiko Yabe
Yoshio Yamaura
Takumi Yoshizawa

From October 1, 2009
Susumu Kumagai, Chairperson
Kosuke Takatori,
Osamu Arakawa
Yasukatsu Oshima
Nobuo Kawahara
Yuko Kumeda
Yukihiro Goda
Yoshiko Konishi
Makoto Shibuya
Yuji Nagashima
Nobuhiro Fusetani
Kimiko Yabe
Yoshio Yamaura
Kanji Yamazaki
Masami Yamada
Takumi Yoshizawa

Executive summary

The Food Safety Commission Japan (FSCJ) conducted a self-tasking risk assessment on deoxynivalenol (DON) and nivalenol (NIV).

The risk assessment was based on scientific data, including in vivo kinetics, acute toxicity, sub-acute toxicity, chronic toxicity, carcinogenicity, reproductive/developmental toxicity, genotoxicity, and immunotoxicity.

Major critical effects of DON derived from animal toxicity studies included emesis, decreased feed intake, suppressed body weight gain, and influence on the immune system. At higher dose levels than those at which the above effects were shown, fetal toxicity and teratogenicity were induced. While weak chromosomal damage was observed in several genotoxicity studies, DON showed no carcinogenic effects on mice in a two-year chronic toxicity study. Based on these results, DON was considered unlikely to have any significant genotoxic activity in vivo. The International Agency for Research on Cancer (IARC) has classified toxins derived from Fusarium species including DON as Group 3, i.e. not classifiable as to its carcinogenicity to humans. The FSCJ has, therefore, concluded that the currently available toxicological data have not sufficiently proved DON to be genotoxic or carcinogenic, and that it is appropriate to establish a Tolerable Daily Intake (TDI) for DON.

After reviewing various toxicity studies, the NOAEL was set at 0.1 mg DON/kg bw/day based on the dose causing suppression of weight gain observed in the two-year chronic toxicity study in mice. By applying an uncertainty factor (UF) of 100 (10 for inter-species differences and 10 for inter-individual variations), the TDI for DON was set at 1 μg/kg bw per day.

Major critical effects of NIV obtained from animal toxicity studies included decreased feed consumption, suppressed body weight gain, and impact on the immune system. Embryotoxicity was observed at doses greater than the dose producing such effects. Although chromosomal damage was reported in some genotoxicity studies, the available data were considered to be inadequate to assess the genotoxic properties of NIV. Since NIV showed no carcinogenic effects in a two-year chronic toxicity study in mice, and the IARC has classified toxins produced by Fusarium species including NIV as Group 3, the FSCJ concluded that a TDI can be set for NIV.

Various toxicity studies have been reviewed, and the LOAEL was set at 0.4 mg NIV/kg bw/day based on the decreased WBC counts observed in sub-acute toxicity study in rats with 90-day oral administration. By applying a UF of 1,000 (10 for inter-species differences, 10 for inter-individual variations, and 10 for the adopted LOAEL value derived from sub-acute toxicity study), the TDI for NIV was set at 0.4 μg/kg bw/day.

Establisment of a group TDI for DON and NIV was considered difficult at present, due to the limited number of studies and varied test results on the combined effects of the two toxins, and the fact that the mechanism of action of each toxin has not been fully clarified.

Estimates of exposure to DON and NIV in Japan were considered below the established TDIs (1 μg/kg bw/day for DON; and 0.4 μg/kg bw/day for NIV). Therefore, dietary intake of DON and NIV was considered unlikely to cause adverse effects on health in the general population of Japan.

I. Background

1. Self tasking assessment and History of selection

The Food Safety Commission conducts risk assessment in response to requests from risk management organizations, and moreover, it implements self-tasking risk assessments taking into account public oionions and expert views.

Candidate substances for FSCJ's self-tasking risk assessments are selected by the Planning Expert Panel based on priority in risk assessment on food, from among substances which are considered to have a major influence on public health, or which highly necessitate the identification of hazards, or whose assessment needs are particularly high. Substances for discretionary assessments are then determined by the FSCJ, after collecting opinions and information from the public and following other relevant procedures.

In March 2009, the FSCJ determined that "ochratoxin A", "deoxynivalenol and nivalenol", and "arsenic (organic and inorganic arsenic) in food" would be subject to its self-tasking risk assessment on food, and that "ochratoxin A" and "deoxynivalenol and nivalenol" would be investigated and discussed by the EPMNT. Based on the opinion of the EPMNT, it was decided that "deoxynivalenol and nivalenol" would be the first to be examined and discussed, since at the 9th Meeting of the EPMNT held on 14 October 2008, a lack of genotoxicity data on ochratoxin A was pointed out, and while at that time, a research on ochratoxin A including collection of such data was underway.

2. Current Regulations etc.

(1) Domestic regulations

In Japan, a provisional regulatory limit of 1.1 mg/kg has currently been set for deoxynivalenol (DON) in wheat (Department of Food Safety Notification No. 0521001, MHLW, 2002). With regards to livestock feed, provisional permissible limits of 4.0 mg/kg (in feed given to cattle 3 months of age or older) and 1.0 mg/kg (in feed given to livestock other than cattle 3 months of age or older) have been set (Agricultural Production Bureau/Livestock Industry Department Notification No. 14-2267, by the Director of Livestock Feed Division, MAFF, 2002).

There are currently no regulatory limits set for nivalenol (NIV).

In addition, the code of practice titled "Guidelines for Reduction of Deoxynivalenol and Nivalenol Contamination in Wheat and Barley" (Joint FSCAB Notification No. 20-8915 and APB Notification No. 20-5731, by the Director-General of Food Safety and Consumer Affairs Burea and by the Director-General of Agricultural Production Bureau, MAFF, 2008) has been established, and consequently, measures to reduce contamination are under way.

(2) Regulatory policies/ guidelines in other countries

The Codex Alimentarius Commission has set no limits for DON or NIV.

The regulatory or guideline limits for DON in food set by different countries are as shown in Figure 1. On the other hand, no country regulates NIV. DON was mostly not regulated as of 1995, but became a matter of great concern for regulatory authorities in and after the latter half of the 1990s, when contamination in the order of several mg/kg was reported in cereals and cereal products in Europe. EU countries have employed a regulatory limit of 750 μg/kg for DON, which has been applied to raw material for wheat flour over the past several years (1).

The U.S. has set a regulatory limit of 1,000 μg/kg for DON in final wheat products. Table 1 shows regulatory limits for DON in cereals and individual cereal products set by the EU (2).

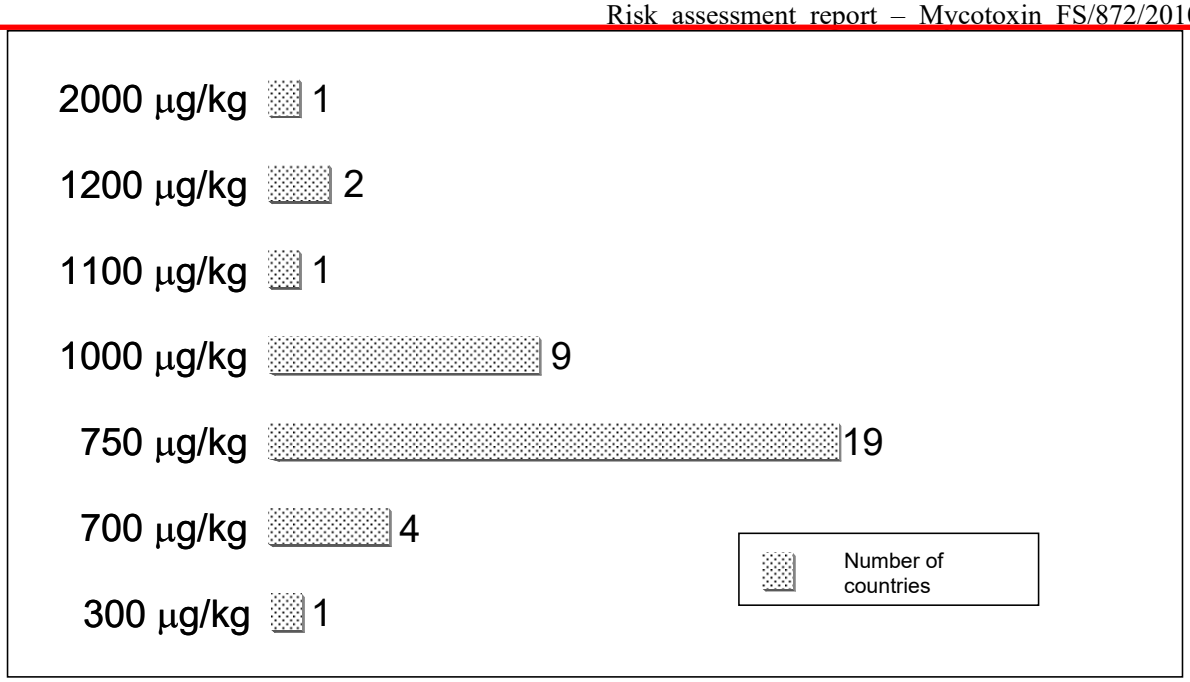


Figure 1. Distribution of regulatory limits for deoxynivalenol (DON) in wheat (flour) or cereals in different countries

Table 1. Regulatory limits for deoxylnivalenol (DON) in EU countries (EU Regulation No.1881/2006)

Food	Maximum levels (μg/kg)
Unprocessed cereal (excluding durum wheat, oats and corn)	1,250
Unprocessed durum wheat and oats	1,750
Unprocessed corn (excluding products for wet milling)	1,750
Cereals and cereal flours for direct consumption (excluding processed cereals for infants)	750
Pasta (dried)	750
Bread, pastries, biscuits, cereal snacks and breakfast cereals	500
Processed cereals for infants	200
Corn flour not for direct consumption (>500 μm in diameter)	750
Corn flour not for direct consumption (≤500 μm in diameter)	1,250

Note: No regulatory limits have been set for rice or rice products.

II. Outline of the Substances under Assessment

1. Name, molecular formula, molecular weight and structural formula

DON and NIV belong to the type B trichothecenes, which are epoxy-sesquiterpenoids. Most trichothecenes have double bonds at C-9 and C-10, a 12,13-epoxy ring, and a number of hydroxyl and acetoxyl groups. Of these, those with a carbonyl group at C-8 are the type-B trichothecenes. (3, 4)

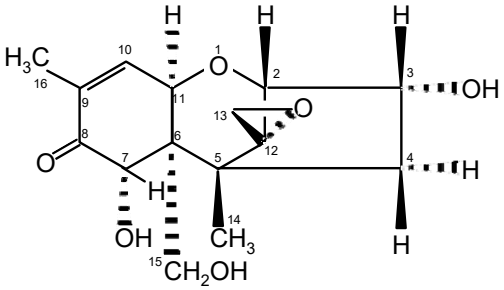
(1) Deoxynivalenol (DON)

(i) Chemical name
CAS (No.51481-10-8)
IUPAC[1]: Trichothec-9-en-8-one, 12, 13-epoxy-3, 7, 15-trihydroxy-(3α,7α)-I
Chem. Absbr. name: 12,13-epoxy-3α,7α,15-trihydroxytrichothec-9-en-8-one

(ii) Molecular formula: $C_{15}H_{20}O_6$

(iii) Molecular weight: 296.32

(iv) Structural formula:



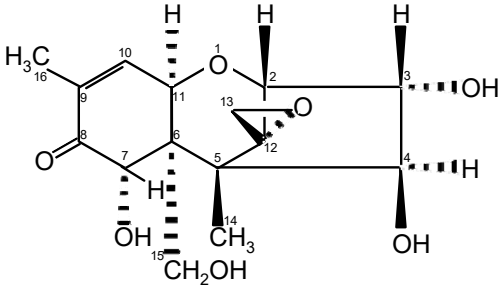
(2) Nivalenol (NIV)

(i) Chemical name
CAS (No.23282-20-4)
IUPAC: Trichothec-9-en-8-one, 12, 13-epoxy-3, 4, 7, 15-tetrahydroxy-(3α,4β,7α)-
Chem. Absbr. name: 12,13-epoxy-3α,4β,7α,15-tetrahydroxytrichothec-9-en-8-one

(ii) Molecular formula: $C_{15}H_{20}O_7$

(iii) Molecular weight: 312.32

(iv) Structural formula:



[1] These substances were named based on the IUPAC's semisystematic nomenclature system under which a compound may be named after the relevant natural substance.

2. Physiochemical properties

Physiochemical properties are summarized below. (4)

(1) Deoxynivalenol (DON)

(a) Appearance: White needle-like crystal
(b) Melting point: 151 - 153˚C
(c) Specific rotation: $[\alpha]_D^{25}$ + 6.35˚ (c=0.07: ethanol solution)
(d) Spectroscopic data: IR, UV, MS and NMR spectra have been reported.
(e) Solubility: Soluble in ethanol, methanol, ethyl acetate, water and chloroform.

(2) Nivalenol (NIV)

(a) Appearance: White crystal
(b) Melting point: 222 - 223˚C (for a product dried under low pressure in the presence of diphosphorus pentaoxide)
(c) Specific rotation: $[\alpha]_D^{24}$ +21.54˚ (c=1.3: ethanol solution)
(d) Spectroscopic data: IR, UV, MS and NMR spectra have been reported.
(e) Solubility: Slightly soluble in water. Soluble in organic polar solvents (5).

3. DON- and NIV-producing organisms

DON and NIV are produced by *Gibberella zeae* and its asexual spore-producing anamorph, *Fusarium graminearum*, as well as *F. culmorum* and other Fusarium and related fungi. They are causative agents for fusarium head blight in cereals, particularly wheat, barley and corn (6, 7). These fungi are widely distributed in the natural world, including soil and crops. While *F. graminearum* was formerly regarded as the producing fungus, it has been classified as a species complex and has been subdivided into thirteen species based on the results of molecular phylogenetic analyses (8, 9). Table 2 shows the main fungal species producing DON and/or NIV and the fungal toxins produced by them.

Table 2. Major Fusarium species involved in deoxynivalenol (DON) and nivalenol (NIV) contamination of food

Species	Fungal toxin produced		Major food crops susceptible to contamination	Geographical distribution
	DON[1)]	NIV[2)]		
F.graminearum species complex	+	+	Wheat, barley, rice, corn	Worldwide
F.graminearum[3)]	+	-	Wheat, barley, rice, corn	Temperate zones (particularly colder regions in the Northern Hemisphere): Japan (all parts), Korea, China
F.asiaticum	-	+	Wheat, barley, rice	Temperate zones (particularly warmer regions): Japan (Honshu and southward), Korea, China
F.vorosii	+	-	Wheat	Japan (Hokkaido), Hungary
F.culmorum	+	+	wheat, barley, corn	Temperate zones (particularly colder regions): Europe, Asia, Africa North and South America, Oceania
F.crookwellense	-	+	Wheat, barley, corn	Temperate zones (particularly colder regions): Japan (Hokkaido)
F.equiseti	-	+	wheat, barley, corn	Subtropical and temperate zones
F.kyushuense	-	+	wheat, barley, rice	Japan (western part), China
F.poae	-	+	wheat, barley, corn	Temperate zones (particularly colder regions):

				Japan (Hokkaido)
F.pseudograminearum	+	-	wheat, barley	Mainly Australia

1) DON: Including DON, 3-acetyl-DON (3-AcDON)[2] and 15-acetyl-DON (15-AcDON)[2].
2) NIV: Including NIV and 4-acetyl-NIV (fusarenon-X, 4-AcNIV)[2].
3) *F.graminearum* s.str. (in a narrow sense)

Fusarium head blight in wheat and barley tends to occur in highly susceptible cultivars. Spores enter wheat or barley heads during the flowering period, and the disease spreads under wet weather conditions (10). Investigations in East Asian countries, including Japan, Korea and China, have reavealed that the main DON-producing and NIV-producing fungi are *F. graminearum* (lineage 7) and *F. asiaticum* (lineage 6), respectively.While both species are distributed mainly in the temperate zones, geographically *F. graminearum* and *F. asiaticum* are distributed in colder regions and warmer regions, respectively (11, 12, 13). Investigations in Japan have found that in Hokkaido, DON contamination is caused by *F. graminearum* and *F. vorosii*, and NIV contamination by *F. crookwellense* and *F. poae*. In Honshu and southward, on the other hand, DON contamination is caused by *F. graminearum* and NIV contamination by *F. asiaticum*. Furthermore, in western Japan the causal agents of NIV contamination also include *F. kyushuense* (11, 14, 15).

4. History of discovery

In Japan, in the 1950s frequent outbreaks of acute Fusarium toxicosis affected both human and livestock, consuming rice, wheat or barley affected by Fusarium head blight. In order to elucidate the causal toxins produced by *F. graminearum*, collaborative studies were organized, for which experts in mycology, chemistry and toxicology were called. This led to the discovery of trichothecene compounds, including NIV and DON (13, 18, 19, 20).

DON was first reported as Rd-toxin, a toxin isolated from *F. roseum* (i.e. *F. graminearum*) which was, in turn, isolated from barley affected by Fusarium head blight that occurred in Kagawa prefecture in 1970 (21). In 1973, the chemical structure of this toxin was first determined in Japan, and the toxin was reported as “deoxynivalenol” (22). It was found that this toxin was identical to the toxin which was separately discovered in the U.S. as a causal agent for moldy corn toxicosis (23) and was named vomitoxin after the disease’s characteristic symptom of vomiting (24, 25). Consequently, studies on the toxicity of DON was proceeded furhter, in Japan, in terms of general toxicity as well as of differences from known trichothecenes and its apocalipsis- and emesis-inducing activities in pigs. Later, toxicity studies on DON were actively carried out all over the world, leading to findings on its chronic toxicity, immunosuppressive activity, etc. (20)

NIV was first isolated from *Fusarium nivale* Fn2B in Japan (18). Its chemical structure was determined during the period from 1966 to 1969, together with that of fusarenon-X (4-acetyl-NIV [4-AcNIV]) (26, 27, 28). Subsequently, *Fusarium nivale* Fn2B was recognized as a new species based on the results of molecular phylogenetic analyses, and was named *F. kyushuense* (29).

During the period from the 1970s to 1990s, toxicity studies of NIV were vigorously carried out in Japan, using molecular toxicological and other advanced methods. These studies led to the establishment of the mechanism of cytotoxicity, such as the induction of apoptosis, and determined future directions for subsequent studies (30).

[2] Types of analogs and their quantitative ratios are different in different strains. Various genes have been reported to be involved in the process of analog production (16, 17).

III. Outline of toxicological Findings

Major safety-related scientific findings are summarized below, based on published literature as well as materials issued by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (2001) (3), the EU Scientific Committee on Food (SCF) (1999, 2000, 2002) (31, 32, 33), and the International Agency for Research on Cancer (IARC) (1993) (4).

1. Disposition in experimental animals

A. Deoxynivalenol (DON)

(1) Absorption, distribution, metabolism and excretion

(i) Conversion to de-epoxy DON in gastrointestinal tract

DON was reported to be converted to de-epoxy DON firstly in rats (34). It was subsequently discovered that the de-epoxidation is caused by intestinal flora. This conversion is known to reduce the toxicity of DON.

A study in which DON was anaerobically incubated with the cecal contents of male Sprague-Dawley rats, showed that de-epoxy DON was gradually formed immediately after the start of incubation, and that 90% of the toxin was converted to de-epoxy DON after 24 hrs of incubation (35).

The conversion of DON by intestinal flora was investigated using swine duodenal, jejunal, cecal, colonic and rectal contents in an *in vitro* study. The highest de-epoxidation activity was found in the colonic contents, where only 1% of the applied toxin was collected in the form of unchanged DON (36).

In another study, DON was not de-epoxidized after 96 hrs of anaerobic incubation with swine large intestinal contents, whereas nearly 100% and 35% of the toxin were converted to its de-epoxy metabolite in the presence of chicken intestinal contents and bovine ruminal fluid, respectively (37).

DON is known to be de-epoxidized by Eubacterium sp. Based on this observation, microbial feed additives containing a Eubacterium strain (BBSH 797) have been used in non-EU European countries, Middle East, Asia and South America (38).

In a study in which ^{14}C-DON was given intragastrically to pigs at a dose of 0.60 mg/kg bw, no conversion of DON was observed (39).

When 3-acetyl-DON (3-AcDON) was ianaerobically incubated with pig feces, the compound was deacetylated into DON, which was further de-epoxidized*in vitro*. The feces from pigs lacking deacetylation ability got the deacetylation ability one week after the pigs were kept in pens, where the feces from pigs known to have the deacetylation ability had been spread out. (40).

After anaerobic incubation of DON with ruminal fluid of female cattle, approximately 80% of the toxin was de-epoxidized (41).

When feed containing DON at a concentration of 8.21 mg/kg dry matter was given to cows, DON was almost completely transformed to de-epoxy DON (94% - 99%) before reaching the duodenum, independent of the amount of feed consumed (42).

An analysis of the degradation of trichothecenes by chicken intestinal flora *in vitro* showed that DON was de-epoxidized, whereas 3-AcDON and 15-acetyl-DON (15-AcDON) were mainly deacetylated (43).

When 3-AcDON was incubated anaerobically with human feces for 48 hrs, 3-AcDON was converted to DON. No de-epoxy metabolite was found (44).

(ii) Absorption

When male PVG rats received oral administration of ^{14}C-DON at a dose of 10 mg/kg bw, 25% of the administered radiolabel was excreted in the urine over 96 hrs suggesting a higher absorption rate in rats than those in sheep or cattle. Bioavailability was not obtained in this study (45).

When castrated pigs were fed a diet containing DON (4.2 mg/kg feed), DON was rapidly absorbed from the stomach and the proximal small intestine. Serum DON levels peaked at 4.1 hrs postdosing. Half of the

absorbed DON was excreted in 5.8 hrs. De-epoxy DON was found mostly in the distal small intestine in these animals (46).

In a study with intravenous administration of ^{14}C-DON to pigs at a dose of 0.30 mg/kg bw, few DON metabolites or conjugates were found, and bioavailability was estimated to be 55% (39).

In castrated pigs that were fed a diet containing DON at a concentration of 5.7 mg/kg feed either once or for 5 – 8 weeks, the estimated bioavailability of 54% and 89% were obtained, respectively (47).

After sheep received a single oral dose of DON at 5.0 mg/kg bw, DON was detected in blood within 30 min, with a bioavailability of 7.5%. On average, 24.8% of the absorbed DON found in the blood was free form, while the remainder consisted of the de-epoxy metabolite or glucuronate conjugates. The de-epoxy metabolite detected in the plasma represented less than 0.3% and less than 2% of the dose administered orally and intravenously, respectively (48).

When sheep received a single oral dose of DON at 5.0 mg/kg bw, the absorption rate was approximately 7%. An average of 6.9% and 0.11% of the administered dose was recovered from urine (1.3% of which was de-epoxy DON or its conjugates and 5.7% of which was DON or its conjugates) and bile (glucuronate conjugates of de-epoxy DON), respectively (49).

In a study in which cows received oral administration of 920 mg/head of DON, a low bioavailability was observed, although no specific numerical data were obtained (50).

In aother study, the absorption of DON was investigated using a swine gastrointestinal tract model (including the stomach, duodenum, jejunum and ileum) *in vitro*. The results showed that most of DON was absorbed in the jejunal portion (51).

(iii) Distribution

After female B6C3F1 mice received a single oral dose of DON at 5 mg/kg bw, DON levels in plasma, spleen, liver, lung and kidney peaked within 15 – 30 min post-dose by both exposure routes of administration, followed by a 75 – 90% reduction within 120 min post-dose. Concentration of DON in plasma and tissues were 1.5 – 3 times higher after nasal than oral administration (52).

In a study in which weaning (3 – 4 weeks old) and young (8 – 10 weeks old) female B6C3F1 mice received oral gavage at a dose of 5 mg DON/kg bw, plasma DON levels in young mice reached the maximum value of 1.0 μg/mL at 15 min post-dose, whereas those in weaning mice were twice as high at the same time point. The same trend was observed in the organs (53).

After oral exposure of DON to mice at a dose of 5 or 25 mg/kg bw, the tissue and plasma concentration reached the maximum level at 30 min or 1 hr post-dose, respectively, with a rapid clearance following a two-compartment model (54).

After pigs received a single intravenous administration of DON at a dose of 1 mg/kg bw, the distribution to different tissues at 3 hrs post-dose was: 550 ng/g in plasma, 930 ng/g in kidney, 440 ng/g in liver, 330 ng/g in abdominal fat, 130 ng/g in back fat, 140 ng/g in lymph, 78 ng/g in lung, 69 ng/g in adrenals, 74 ng/g in spleen, 54 ng/g in testis, 29 ng/g in brain, 11 ng/g in heart, 19 ng/g in muscles, 16 ng/g in skin, 5 ng/g in intestine, and 4 ng/g in pancreas. The distribution at 24 hrs post-dose was: 18 ng/g in plasma, 10 ng/g in kidney, 8.2 ng/g in liver, 3.4 ng/g in abdominal fat, 12 ng/g in back fat, 0.8 ng/g in lymph nodes, 1 ng/g in lung, and not detected in any of the other tissues (55).

After ^{14}C-DON wasgiven to chickens at a single oral dose of 1.3 – 1.7 mg/kg bw, the distribution to different tissues at 3 hrs post-dose was: 416 dpm/g[3] in blood, 570 dpm/g in plasma, 4,345 dpm/g in bile, 19 dpm/g in cutaneous fat, 10 dpm/g in abdominal fat, 5 dpm/g in breast muscles, 5.3 dpm/g in thigh muscles, 91 dpm/g in spleen, 205 dpm/g in liver, 27 dpm/g in heart, 733 dpm/g in kidney, 21 dpm/g in brain, and 5 dpm/g in oviduct. The average distribution at 72 hrs post-dose was: 0 dpm/g in blood, 0 dpm/g in plasma, 661 dpm/g in bile, 10 dpm/g in subcutaneous fat, 9.8 dpm/g in abdominal fat, 0.5 dpm/g in breast muscles, 2 dpm/g inthigh muscles, 8 dpm/g in spleen, 10 dpm/g in liver, 0 dpm/g in heart, 18 dpm/g in kidney, 0 dpm/g in brain, and 2 dpm/g in oviduct. At 96 hrs post-dose, the radiolabelled compound was detected only in cutaneous fat, kidney, gizzard and bile (56).

(iv) In vivo metabolism

[3] "dpm" stands for disintegration per minute and is calculated by dividing counts per minute (cpm) by counting efficiency.

In a study using rabbit or rat liver microsomal fractions, no metabolism of DON was observed (57, 58).

DON is known to form glucuronate conjugates in cattle (59, 60). In sheep, the formation of glucuronate and sulfate conjugates has been reported (48, 61).

(v) Excretion

When male PVG rats received oral administration of ^{14}C-DON at a dose of 10 mg/kg bw, 25%, 64% and 0.11% of the administered radiolabel were recovered during the first 96 hrs, respectivery from urine, feces and expired air. DON and its de-epoxy metabolite were identified in the urine and feces in this study (45).

In male Sprague-Dawley rats administered ^{14}C-DON by oral gavage at a dose of 5 mg/kg bw, plasma level of ^{14}C-DON peaked at 8 hr, where 9% of the ^{14}C-DON bound to plasma proteins. Thirty-seven percent of the administered ^{14}C-DON was excreted in urine, and the main metabolites found in urine were glucuronate conjugates (62).

In a study in which pigs received intravenous administration of DON at a dose of 1 mg/kg bw, DON was recovered in bile and urine with the elimination half-life of 3.9 hrs (55).

When castrated pigs were recieved feed containing 4.2 mg/kg of DON for 7 days, the proportion of de-epoxy DON increased in the distal portion of small intestine. In feces collected from the rectum, the proportion of de-epoxy DON to the total of DON and de-epoxy DON was approximately 80% (46).

Following intravenous (0.30 mg/kg; 0.35 μCi/kg) or intragastric (0.60 mg/kg; 0.60 μCi/kg) administration of ^{14}C-DON to pigs, 93.6% of the intravenous DON was excreted in urine, whereas 68.2% and 20.3% of the intragastric DON were excreted in urine and feces, respectively (39).

Rapid excretion of DON was shown in chickens received a single oral dose of 2.2 mg ^{14}C-DON (corresponding to 1.3 – 1.7 mg/kg bw), where 79%, 92% and 98% of the administered dose were excreted during the first 24, 48 and 72 hrs post-dose, respectively (56).

It has been reported that in male sheep received a single oral dose of DON at 5 mg/kg bw, DON and de-epoxy DON were eliminated completely from the plasma within 30 hr post-dose (48).

When DON was administered to sheep at a dose of 5 mg/kg bw, 6.9%, 0.11% and 65% of the administered DON were collected from urine, bile and feces, respectively, in the form of unchanged DON or its metabolites (49).

The same group subsequently reported that in female sheep received intravenous administration of ^{14}C-DON at a dose of 4 mg/kg bw, 91% and 6% of the dose were recovered from urine and bile, respectively, during the first 24 hr post-dose (61).

In human, it has been reported that glucuronate conjugates of DON are excreted in urine (62).

(vi) Transfer into eggs and milk

When hens received a single oral dose of 2.2 mg ^{14}C-DON (corresponding to a dose of 1.3 – 1.7 mg/kg bw), the maximum amount of ^{14}C-DON contained in the first egg laid within 24 hrs post-dose was 0.087% of the administered dose (corresponding to 1.9 μg of ^{14}C-DON per egg). After repeated oral administration of ^{14}C-DON for 6 days, the maximum amount of radioactivity per egg was 0.19% of the daily dose (corresponding to 4.2 μg of ^{14}C-DON per egg) (63).

In a study in which chickens were treated with feed containing ^{14}C-DON at a concentration of 5.5 mg/kg feed for 65 days, there was no increase in the amount of ^{14}C-DON accumulated in eggs. The radioactivity in eggs reached the maximum (corresponding to 1.7 μg of DON or its metabolites per 60 g of egg) after 8 days of administration, followed by a gradual decrease over the next several weeks (64).

After female sheep received intravenous administration of ^{14}C-DON at a dose of 4 mg/kg bw and were monitored for the transfer of the compound into milk for 48 hrs, less than 0.25% of the administered dose was recovered. The maximum levels of DON and its de-epoxy metabolite in milk were 61 ng/mL and 1,220 ng/mL, respectively, with the ratios of conjugated to non-conjugated metabolites being approximately 2:1 and approximately 3:1 – 5:1, respectively (61).

In milk collected twice daily from cows that had received a single oral dose of 920 mg DON, only low levels of free and conjugated forms of DON were detected, with the maximum level being 4 ng/mL (50).

Using primiparous Holstein cows at 13 – 22 weeks of lactation, the effects of DON in feed on milk

production as well as on the transfer of DON and de-epoxy DON into milk were studied for 10 weeks. The results indicated that, while the administered doses of DON (corresponding to daily intakes of 0.001, 0.085 and 0.21 mg/kg bw) had no effect on feed consumption or total milk yield, milk fat content and the amount of total milk fat decreased in the two groups treated with DON. No transfer into milk of DON or its de-epoxy metabolite was observed (detection limit: 5 ng/mL) (65).

When cows were fed a diet containing 8.21 mg DON and 0.09 mg zearalenon (ZEN) per kg dry matter respectively, the carry over rates of DON and epoxiy DON into milk (i.e., the proportion of the amount excreted in milk to the DON intake) were ranging from 0.0001 to 0.0002 and 0.0004 to 0.0024, respectively (42).

When holstein cows were fed 5.3 mg DON per kg dry matter for 11 weeks or 4.4 or 4.6 mg DON for 18 weeks, DON was not detected in milk, while de-epoxy DON was detected at concentrations ranging between below the detection limit and 3.2 µg/kg of milk. The carry over rates of DON in milk were negligible, ranging from 0.0001 to 0.0011 (66).

(2) Effects on biochemical parameters

In a study where male NMRI mice were fed a diet containing DON for 6 weeks, the group fed a diet containing 10 mg /kg DON (corresponding to 1.4 mg/kg bw) showed a significant decrease in body weight gain ($p < 0.01$). At the end of the feed period, the absorption of water, leucine, tryptophan or iron was studied using isolated perfused jejunal segments *in vitro*. No effects were observed on the absorption, except a slight decrease in the rate of glucose transfer ($p < 0.05$) in the group given feed containing 10 mg /kg of DON. In addition, the rates of transfer and of tissue accumulation of 5-methyltetrahydrofolic acid in the jejunum decreased by up to 50%. This group showed also low manganese and molybdenum contents in the liver (67).

In a study using spleen segments isolated from 8- to 10-week-old male rats, minimum effective level of DON on protein and DNA synthesis was 1,000 ng/mL (72% and 53%, respectivery). On the other hand, RNA synthesis was promoted at the same level (68).

In *in vivo* and *in vitro* studies, DON suppressed uptake of glucose and amino acids in chicken small intestine by inhibiting the Na^+/D-glucose co-transporter and Na^+/amino acid co-transporter, respectively (69, 70, 71).

After male Wistar rats were administered subcutaneously with 1 mg DON /kg bw once a day for 3 days, the levels of blood insulin, glucose and free fatty acid were increased. In addition, muscle glycogen accumulation was increased and triglycerides content was decreased (72).

Most trichothecenes inhibit protein synthesis. This inhibition requires unsaturated bonds at C-9 and C-10 and a 12, 13-epoxy ring, and inhibition potency depends on the substituents. Trichothecenes bind to the 60S subunit of eukaryotic ribosomes and inhibit peptidyl transferase activity. DON, which lacks substituent at C-4, inhibits peptide-chain elongation (73, 74). The inhibition of protein synthesis is considered as the main toxic activity of trichothecenes, including DON (75). DON is approximately hundred times less toxic than T-2 toxin *in vitro*. Due to differences in lipid solubility and other possible factors, the toxicity of DON *in vivo* is expected to be greater than would be expected from its effects *in vitro* on protein synthesis (75, 76).

A study comparing the cytotoxicity of DON on different cultured cells using MTT assay, an assay used to measure cell proliferation and activities, revealed their susceptibility to DON. The susceptibility was highest in CHO-K1 cells (a Chinese hamster ovary-derived cell line), followed in descending order by V79, C5-O, Caco-2 and HepG2 cells (cell lines derived from Chinese hamster lung cells, BALB/c mouse keratinocytes, the human gastrointestinal tract, and human liver, respectively), with IC_{50} (the concentrations of DON at which the cell growh was inhibited by 50% after 48 hrs of exposure 50% inhibitory concentration;) of 0.27, 0.49, 0.54, 1.02 and 8.36 µg/mL, respectively (77).

After primary rat liver cells were exposed to 10 – 2,500 ng/mL of DON for 24 hr, lactate dehydrogenase, ALT and AST levels were increased and the cell survival rate was decreased. The IC_{50} measured by MTT assay in this study was 1,200 ng/mL, and morphological damage was observed at concentrations of 10 ng/mL and above. The cytotoxic effects were dose dependent and had a threashold value of 50 ng/mL (78).

When HuH-6KK cells (a human liver-derived cell line) were incubated in serum-free medium containing 0.15 mg/L each of DON, acetyl-NIV (AcNIV) and NIV, cell proliferation was inhibited by those fusarium

toxins. The IC_{50} of DON measured by MTT assay was 1.1 mg/L (79, 80).

Cytotoxicity was compared between DON and its glucuronate conjugates using K562 cells (a human erythroleukemia cell line), by means of bioassay using MTS for measuring cell proliferation and activities. The results showed a 50% inhibition of cell growth (activity) at 1.31 μM of DON, while glucuronate conjugates showed no significant cytotoxicity up to 270 μM (81).

When the effects of DON, 3-AcDON, 15-AcDON and de-epoxy DON on the proliferation of 3T3 cells (a mouse skin-derived cell line) were examined by 5-bromo-2'-deoxyuridine (BrdU) uptake assay, the IC_{50} values were 1.50 ± 0.34 mM (444 ± 101 ng/mL), 14.4 ± 1.59 mM (4,890 ± 537 ng/mL), 1.51 ± 0.24 mM (510 ± 80 ng/mL) and 83.0 ± 8.77 mM (23,300 ± 2,460 ng/mL), respectively (82).

DON (10 – 100 μM) induced dose-dependent apoptosis in J774A.1 cells (a mouse macrophage-like cell line), with the IC_{50} of 16.8 ± 0.2 μM after 72 hrs of incubation (83).

In an *in vitro* study where the biotransformation of DON was using swine intestinal contents was investigated, a correlation between the de-epoxidation of DON and the reduction of cytotoxicity was observed using swine kidney cells by MTT assay (36).

The above findings suggested that, while there are variations depend on animal species and doses, DON was converted and metabolized into less toxic derivatives mainly by de-epoxidation and glucuronate conjugation, followed by excretion in urine and feces together with unchanged DON (Fig. 2).

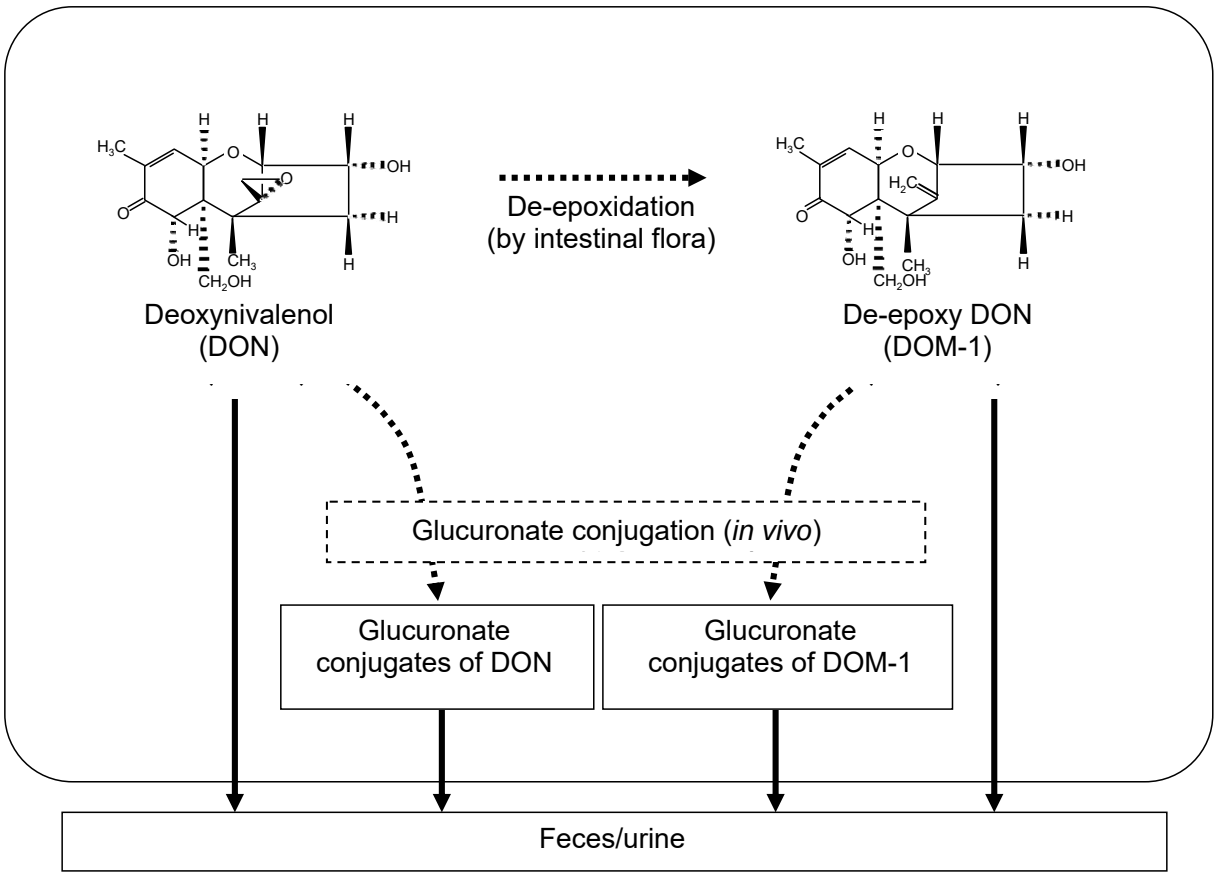


Fig. 2 Summary of major conversion and metabolic pathways of deoxynivalenol (DON)

B. Nivalenol (NIV)

(1) Absorption, distribution, metabolism and excretion

(i) Conversion to de-epoxy NIV in gastrointestinal tract

NIV is known to be converted to less toxic derivatives through de-epoxidation by intestinal flora.

Anaerobical incubation of NIV with swine feces *in vitro* was shown to de-epoxidize NIV. When feces from pigs with a de-epoxidation activity was sprayed over a pig-cote housing pigs, lacking the metabolic activity of de-epoxidation, feces from these pigs obtained de-epoxydation activity in one week (Ref. 40).

However, *in vitro* anaerobic incubation of feces from pigs with NIV, prior to the NIV exposure period, failed to result in de-epoxidation of NIV. On the other hand, when pigs were fed with diet containing 2.5 or 5.0 mg/kg of NIV for 1 week, their intestinal flora obtained the activity to de-epoxidize NIV, and *in vitro* incubation of DON with feces of these animals resulted de-epoxydation of DON. After *in vitro* anaerobic incubation of NIV with ruminal fluid of cattle, approximately 80% of the toxin was de-epoxidized (41).

(ii) Absorption

When female ICR mice were orally exposed to ^{3}H-NIV or ^{3}H-AcNIV at doses of 20 or 18 μg/kg bw, respectively, the plasma levels of ^{3}H-NIV or ^{3}H-AcNIV peaked at 60 or 30 min after administration, respectively. The maximum plasma level and AUC in ^{3}H-AcNIV administrated mice were 5-fold and 10-fold higher than those in ^{3}H-NIV administrated mice, respectively. After being absorbed, AcNIV was rapidly metabolized to NIV in the liver and kidneys (84).

The absorption of NIV was studied in pigs fed NIV at a dose of 0.05 mg/kg bw twice a day. Blood samples were collected via catheters placed in the hepatic portal vein and the peripheral mesenteric artery. NIV was found to be absorbed from the intestines, and was detected in the blood starting from the first sampling time (i.e., 20 min post-dose). Eleven to 48% of the administered dose was absorbed within the first 7.5 hrs post-dose, with plasma NIV levels peaking at 2.5-4.5 hrs post-dose (85).

AcNIV was immediately converted to NIV after AcNIV was administered intravenously or orally to broilers and ducks at a dose of 2.2 mg/kg bw. Following the intravenous administration, NIV was detected in the blood immediately post-dose and remained at high levels until 20 min post-dose. After the oral administration, blood AcNIV and NIV levels peaked at 10 min post-dose, with a major part of AcNIV having immediately been converted to NIV. The bioavailability[4] of AcNIV after oral administration was 9.8% and 19.5% in broilers and ducks, respectively (86).

The absorption of NIV was investigated using an *in vitro* swine gastrointestinal tract model (including the stomach, duodenum, jejunum and ileum). The results showed that most of the toxin was absorbed in the jejunal portion (45).

In an *in vitro* study of the degradation of trichothecenes by chicken intestinal flora, NIV was de-epoxidized while AcNIV was mainly de-acetylated (43).

An *in* vitro experiment using Caco-2 cells showed that the basal-to-apical transport of NIV is energy dependent, while its apical-to-basal transport is by simple diffusion (87).

(iii) Distribution

In ofer to investigate the transfer of NIV and AcNIV from pregnant of fegal mice, ICR mice at day 17 of gestation were given ^{3}H-NIV and ^{3}H-AcNIV at doses of 40 or 43 mg/kg bw, respectively. In the mother mice, the radioactivity was found to be distributed in the plasma, liver, kidney and placenta at both 6 and 24 hrs post-dose. In their fetuses, the radioactivity was found in all organs, including the liver and kidney, at and after 6 hrs post-dose and at appoximately the same levels as their mothers' (88).

(iv) In vivo metabolism and excretion

In a study in which rabbit or rat liver microsomal fractions, on metabolism of NIV was observed (57).

In a study in which female ICR mice were administered orally with ^{3}H-NIV or ^{3}H- AcNIV at doses of 20 or 18 μg/kg bw, respectively, the radioisotope was excreted in urine or mainly in feces in mice given ^{3}H-AcNIV and in those given ^{3}H-NIV, respectively, at 48 h post-dose (84).

After male Wistar rats received oral administration of NIV in a total of 12 doses of 5 mg/kg bw at an interval of 2-3 days, 80% and 1% of the administered NIV were excreted as de-epoxy NIV in feces and urine, respectively. Seven percent and 1% of the administered dose were detected as unchanged NIV in feces and urine, respectively (89).

[4] Bioavailability is expressed as a percent and reprsents the proportion of the total amount of the unchanged compound in circulation to the administered dose.

After pigs were given feed containing NIV at a dose of 0.05 mg/kg bw twice a day, NIV was excreted mainly in feces. None of the NIV metabolites (i.e., glucuronate and sulfate conjugates and de-epoxy NIV) was found in the plasma, urine or feces (85).

In a study in which female chickens were fed diets containing NIV at concentrations of 1, 3 and 5 mg/kg for 50 days, trace amounts of unchanged NIV were found in the liver and bile. Up to 10% of the intake of NIV was excreted unchanged or as de-epoxy NIV in feces (90).

(v) Transfer into eggs and milk

In order to study the transfer of NIV from lactating to suckling mice, lactating ICR mice were orally given ^{3}H-NIV or ^{3}H-AcNIV at a dose of 40 or 43 mg/kg bw, respectively, by oral gavage, and the radioactivities in milk and tissue were mesured at 6 and 24 hrs. Radioactivity was detected in the milk as well as in liver and kidney of suckling mice. A HPLC analysis of the compounds demonstrated that AcNIV is converted mainly to NIV in mother mice's body, followed by transfer into sucklings (88).

(2) Effects on enzymes and other biochemical parameters

NIV inhibited the proliferation of HeLa cells (a human uterus-derived cell line) totally at a concentration of 0.5 μg/mL. At 5 μg/mL, NIV almost completely inhibited protein and DNA synthesis, but scarcely inhibited RNA synthesis (91).

After HeLa cells were treated with NIV at a concentration of 15 μg/mL for 1 min, no inhibition of RNA synthesis was observed, while polyribosome degradation was induced (92). NIV also inhibited the proliferation of other human-derived cells (uterine cancer and fetal kidney cells and lymphocytes), with the IC_{50} of 0.3 – 1.0 μg/mL (93).

When rabbit reticulocytes were treated with NIV, protein synthesis was inhibited with an IC_{50} of 6 μg/mL. The IC_{50} of 0.5 μg/mL for the inhibition of polyphenylalanine synthesis suggests that NIV inhibits protein synthesis at the ribosome level (94). In Ehrlich ascites tumor cells, NIV inhibited protein synthesis (IC_{50}, 6 μg/mL) and DNA synthesis (IC_{50} > 10 μg/mL) (95).

NIV (10 – 100 μM) induced concentration-dependent apoptosis in J774A.1 cells, with the IC_{50} after 72 hrs of incubation being 11.2 ± 0.8 μM (83).

When the effects of NIV, 4-AcNIV and de-epoxy NIV on the proliferation of 3T3 cells were studied using BrdU intake test, the IC_{50} values were 1.19 ± 0.06 mM (373 ± 20 ng/mL), 0.72 ± 0.04 mM (255 ± 13 ng/mL) and 64.2 ± 3.14 mM (19,030 ± 930 ng/mL), respectively (82).

In oder to stucy the effects of NIV on the hepatic drug metabolism in mice, male C57B16 mice were given orally NIV three times per week for a four weeks at doses of 0.014, 0.071, 0.355, 1.774 or 8.87 mg/kg bw. No change in P450 1a, 2b, 2c, 3a or 4a expression was observed by Western blot analysis (Ref. 96).

The above findings suggest that, while there are variations depending on animal species and doses, NIV is converted to a less toxic derivative mainly through de-epoxidation by intestinal flora. This derivative is excreted in urine and feces together with unchanged NIV. AcNIV is converted and metabolized to NIV mainly through deacetylation (Fig. 3).

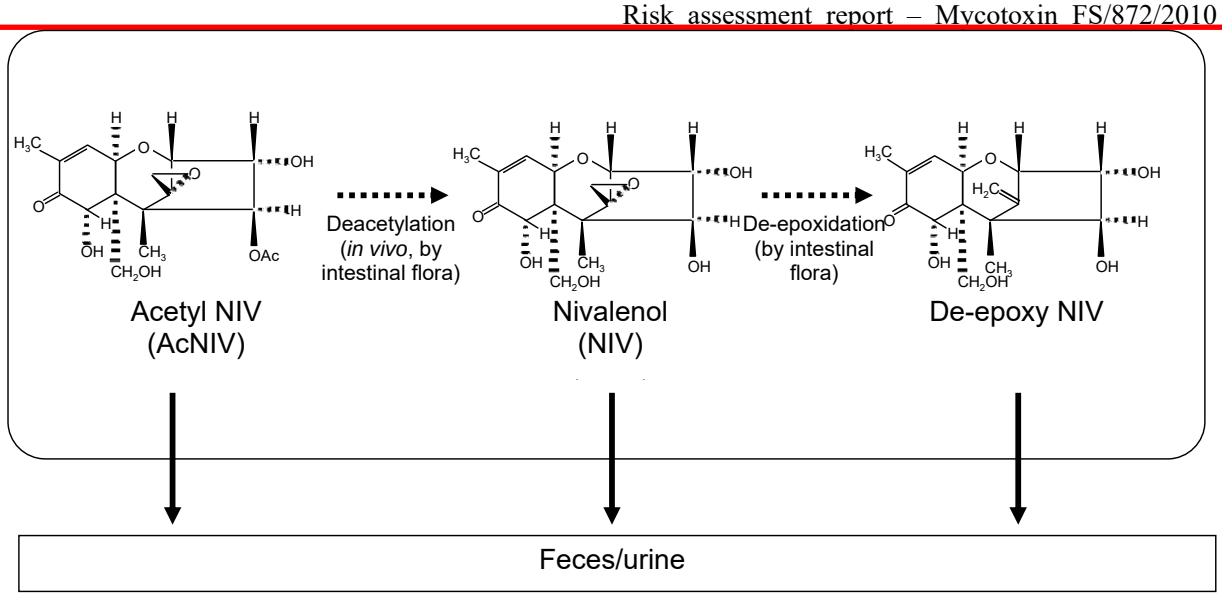


Fig. 3 Summary of major conversion and metabolic pathways of nivalenol (NIV)

2. Toxicity in experimental animals

In compiling toxicity data, data from studies emplying the administration of purified DON or NIV were basically used, in order to clarify toxicological findings specific to the administration of DON or NIV. Experiments with the administration of naturally contaminated feed or other materials that might have been contaminated by other toxin(s) were also taken into account if necessary. Since the targets of the present assessment are DON and NIV present in food, data were compiled also from studies with oral administration..

A. Deoxynivalenol (DON)

(1) Acute toxicity

Table 3 shows the 50% lethal dose (LD_{50}) after oral administration of DON. Toxicological changes after a single oral dose of DON are characterized by damages to the gastrointestinal tract and lymphoid tissues as well as by emesis.

Table 3 LD_{50} in acute oral toxicity of deoxynivalenol (DON)

Species and strain	Substance administered	LD_{50} (mg/kg bw)	Ref.
Mouse, DDY, male, 6-week old	Purified DON	46	97
Mouse, B6C3F1, female, weanling	Purified DON	78	98
Chicken, male, 1-day old	Purified DON	140	99

LD_{50} of purified DON after the oral administration to mice have been reported to be 46 (97) and 78 mg/kg bw (98), with marked gastrointestinal (GI) bleeding, and necrosis of bone marrow and kidney.

In an experiment in which B6C3F1 mice (three females per group) received a single oral dose of DON, extensive necrosis of the GI tract, bone marrow and lymphoid tissues were observed at and above the dose of 100 mg/kg bw (98). In an experiment using DDY mice (10 males per group), gastric fundal hemorrhage, subarachnoid hemorrhage and testicular hyperemia were observed after the administration of DON at and above 32 mg/kg bw (97).

In a single dose experiment in pigs, the administration of DON at 0.4 mg/kg bw affected the duodenum (mucosal hyperemia and edema), jejunum (hyperemia in the villi, eosinophilic infiltration and enlarged lymphoid follicles), ileum (enlarged lympohid follicles) and liver (vacuolar degeneration and necrosis of

liver cells, and hyperemia) (100).

Table 4 shows a summary of events of emesis observed in expermiental animals treated with DON. The emetic effect seems to work through the nervous system, since intravenous and intraperitoneal administration induces emesis at the same dose levels as oral administration.

The minimum emetic dose was 0.05 – 0.1 mg/kg bw in pigs received a single oral gavage. On the other hand, emesis did not occur up to 0.19 – 0.6 mg/kg bw per day, when pigs were given feed containig DON. In dogs, emesis occurred after subcutaneous administration of purified DON at a dose of 0.1 mg/kg bw, while no emesis was found up to 0.45 mg/kg bw per day after administration with feed (97, 112). In sheep and pigs received intravenous adminitration of DON at a dose of 1.0 mg/kg bw, DON was detected in the cerebrospinal fluid. In pigs, the amount of DON reaching the cerebrospinal fluid was found to be approximately 2.5-fold compared with in sheep (113). It has been reported that administration of a serotonin receptor ($5HT_3$: 5-hydroxytryptamine, type3) antagonist prevented DON-induced emesis in pigs (103). DON has also been reported to inhibit small intestinal motility through $5HT_3$ receptors in rodents, with such findings as gastric relaxation and delayed gastric emptying (114).

Table 4 Summary of emesis in experimental animals administered with deoxynivalenol (DON)

Description of Target (Number per group).	Route (solvent) Duration of dosing	Substance administered	Dose	Effects	ED_{50} (mg/kg bw)	Minimum emetic dose (mg/kg bw)	Maximum non-emetic dose (mg/kg bw)	Reference
Pig, crossbred, 9-10 kg (3- 6/ group)	Oral gavage (water), single dose	Purified DON	0, 0.075, 0.1, 0.2, 0.4 mg/kg bw	- At 0.1 mg/kg bw, 1 of the 6 animals had emesis at 82 min post-dose. - At 0.2 mg/kg bw, 2 of the 3 animals had emesis at an average of 68.5 min post-dose. - At 0.4 mg/kg bw, all 3 animals had emesis at an average of 59 min post-dose.		0.1	0.075	101
	Intraperiton-eal, single dose	Purified DON	0, 0.025, 0.05, 0.075, 0.1, 0.2 mg/kg bw	- At 0.05 mg/kg bw, 2 of the 3 animals had emesis. - At and above 0.075 mg/kg bw, all 3 animals had emesis.		0.05	0.025	
Pig, Yorkshire, 10-15 kg (3 /group)	Oral gavage (saline), single dose	Purified DON	0, 0.025, 0.05, 0.075, 0.1, 0.2 mg/kg bw	- At 0.05 mg/kg bw, 1 of the 3 animals had emesis at 56 min post-dose for a duration of 14 min. - At 0.075 and 0.1 mg/kg bw, no emesis occurred. - At 0.2 mg/kg bw, all 3 animals had emesis at an average of 19.3 min post-dose for an average duration of 16.3 min.		0.05	0.025	102
	Intraperiton-eal (saline), single dose	Purified DON	0, 0.025, 0.05, 0.075, 0.1, 0.2 mg/kg bw	- At 0.05 mg/kg bw, 1 of the 3 animals had emesis. - At 0.075 and 0.1 mg/kg bw, all 3 animals had emesis. - At 0.2 mg/kg bw, 2 of the 3 animals had emesis.		0.05	0.025	
	Oral gavage (saline), single dose	Purified 15-AcDON	0, 0.025, 0.05, 0.075, 0.1, 0.2 mg/kg bw	- At 0.075 mg/kg bw, 1 of the 3 animals had emesis. - At 0.1 mg/kg bw, no emesis occurred. - At 0.2 mg/kg bw, 2 of the 3 animals had emesis.		0.075	0.05	
	Intraperiton-eal (saline), single dose	Purified 15-AcDON	0, 0.025, 0.05, 0.075, 0.1, 0.2 mg/kg bw	- At and above 0.075 mg/kg bw, all 3 animals had emesis.		0.075	0.05	
Pig, Yorkshire, 6 -8-week-old, 15-20 kg (4-6 /group)	Intragastric (DMSO), after 4 hrs of fasting, single dose	Purified DON			0.075			103
	Intravenous, single dose	Purified DON			0.02			
Pig, Yorkshire, neutered male,	Intragastric (saline), 6 doses at 30 min intervals	Purified DON	0, 0.03 mg/kg bw	- No emesis			0.03	104

Description of Target (Number per group).	Route (solvent) Duration of dosing	Substance administered	Dose	Effects	ED_{50} (mg/kg bw)	Minimum emetic dose (mg/kg bw)	Maximum non-emetic dose (mg/kg bw)	Reference
8-12-week-old, 15-20 kg (2-4 /group)	Intravenous (saline), 6 doses at 30 min intervals	Purified DON	0, 0.01 mg/kg bw	- No emesis			0.01	
Pig, Yorkshire, neutered male, 8-12-week old, 15-20 kg (2-4/group)	Intragastric (saline), single dose	Purified DON	0, 0.03, 0.3 mg/kg bw	- At 0.3 mg/kg bw, all 4 animals had emesis within 15 min.		0.3	0.03	105
	Intravenous (saline), single dose	Purified DON	0, 0.01, 0.1mg/kg bw	- At 0.1 mg/kg bw, all 4 animals had emesis within 15 min.		0.1	0.01	
Pig, crossbred, 20 kg (4/group)	Diet, 4 days	Purified DON	0, 3.6, 7.2, 40 mg/kg feed	- No emesis				101
Pig, Yorkshire, neutered male, 9-10-week-old, 27.5 kg (3/group)	Diet, 49 days	Purified DON	0, 4.7 mg/kg feed (0.19 mg/kg bw/day*)	- No emesis			0.19*	106
Pig, 7.5 kg (4/group)	Diet, 4 days	Artificially contaminated corn	0, 44.4, 97.2, 124.9, 227.5 mg/kg feed	- At 44.4 mg/kg feed, 2 of the 4 animals had emesis - At 97.2 mg/kg feed, 1 of the 4 animals had emesis - At 124.9 mg/kg feed, all 4 animals had emesis. - At 227.5 mg/kg feed, 3 of the 4 animals had emesis.				107
Pig, 8.4 kg (4/group)	Diet, 11 days	Artificially contaminated corn	0, 9.0, 19.7, 33.5, 43.4 mg/kg feed	- At and above 19.7 mg/kg feed, emesis occured on Day 1.		0.8*		
Pig, 7.1 kg (3/group)	Diet, 21 days	Artificially contaminated corn	0, 1.34, 2.55, 5.12, 6.39, 7.83, 8.63, 11.9 mg/kg feed	- No emesis			0.6*	

Description of Target (Number per group).	Route (solvent) Duration of dosing	Substance administered	Dose	Effects	ED_{50} (mg/kg bw)	Minimum emetic dose (mg/kg bw)	Maximum non-emetic dose (mg/kg bw)	Reference
Pig, Yorkshire, neutered male and nulliparous female, 34-39 kg (5 each sex/group)	Diet, 5 weeks	Artificially contaminated corn or naturally contaminated wheat	0, 5.08, 14.5 mg/kg feed (0, 0.2, 0.42 mg/kg bw/day)	- No emesis			0.42	108
Pig, 74 kg (64 females/ group)	Diet, 35 days	Contaminated wheat	0, 5 mg/kg feed	- No emesis				109
Pig, weanling, 7.7 kg (8 each sex/group)	Diet, 3 weeks	Contaminated wheat	0, 0.9, 2.0, 2.8 mg/kg feed	- No emesis				110
Pig, 23-27 kg (15/group)	Diet, 9 weeks	Naturally contaminated corn	1, 5mg/kg feed	- At 5 mg/kg feed, emesis occurred.				111
Dog, 6-month old, 2-3 kg (5-7/group)	Subcutane-ous, single dose	Purified DON	0, 0.025, 0.1, 0.2, 0.5, 1.0, 2.0, 3.8 mg/kg bw	- At 0.1 - 0.2 mg/kg bw, emesis occurred at 10-odd min post-dose. - At 1 - 2 mg/kg bw, emesis occured at several min post-dose.		0.10	0.025	97
Dog, Beagle or Brittany, 1-7-year old, 15-20 kg (2-14/group)	Diet, 14 days	Naturally contaminated wheat	0, 1, 2, 4, 6, 8, 10 mg/kg feed (0, 0.075, 0.15, 0.3, 0.45, 0.6, 0.75 mg/kg bw/day*)	- At and above 8 mg/kg feed, emesis occurred.		0.6*	0.45*	112
Cat, American Shorthair, 1-9-year-old, 2-4 kg (2-8/group)	Diet, 14 days	Naturally contaminated wheat	0, 1, 2, 4, 6, 8, 10 mg/kg feed (0, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5 mg/kg bw/day*)	- At 4 mg/kg feed, 1 of the 2 animals had emesis. - At 6 and 8 mg/kg feed, no emesis occurred. - At 10 mg/kg feed, 4 of the 8 animals had emesis.		0.2*	0.1*	112

*: Converted values based on the JECFA standards.

(2) Subacute toxicity

Table 5 shows the results of subacute toxicity studies of DON.

Table 5 Results of short-term diet studies of the toxicity of the purified deoxynivalenol (DON)

Description of Target (Number per group).	Route (solvent) Duration of dosing	Dose (mg/kg feed)	Dose (mg/kg bw/day)	Effects	LOAEL (mg/kg bw/day)	NOAEL (mg/kg bw/day)	Comments	Reference
Mouse, BALB/c, 4-6-week-old (4 males /group)	7 days	0,2.5,5,10,20, 50	0, 0.35, 0.67, 1.3, 2.7, 6.5	- At and above 2.5 mg/kg feed, decreased feed consumption. - At and above 10 mg/kg feed, decreased weight gain and decreased thymus weight.	1.3	0.67	Decreased indicator weight	115
	30 days	0, 10–20		- After 2-3 weeks of treatment, 3 of the 4 animals developed endocarditis legions with calcification.				
Mouse, ICR, 3-week -old (10 each sex/group)	14 days	0,2,4,8	Males: 0, 0.37, 0.76, 1.49. Females: 0, 0.41, 0.81, 1.59.	- At 8 mg/kg feed, decreased feed consumption. - At and above 2 mg/kg feed, decreased weight gain (in males) and decreased RBC count.	0.37			116
Mouse, ICR, 3-week old (10-12 females /group)	14 days	0,8,12,16	0, 1.2, 1.8, 2.4	- Dose-dependent decrease in weight gain and feed consumption.	1.2			117
		0,4,8	0, 0.6, 1.2	- At and above 4 mg/kg feed, decreased weight gain.	0.6			
Mouse, Swiss-Webstar, weanling (24 males /group)	35 days		0, 0.75, 2.5, 7.5	- In the 7.5 mg/kg bw group, 23 of the 24 animals died during the study period. - In the 2.5 mg/kg bw/day group, 12 of the 24 animals died. - At and above 2.5 mg/kg bw/day, changes in the spleen, thymus, lymph nodes and gastrointestinal tract. - At and above 0.75 mg/kg bw/day, decreased body weight and feed consumption.	0.75			118
Mouse, NMRI, 18 g (10 males /group)	42 days	0.1, 1, 10	0.014, 0.14, 1.4*	- At 10 mg/kg feed, suppressed weight gain and impaired nutrient intake.	1.4*	0.14*		67
Mouse, B6C3F, weanling (8 females /group)	56 days	0, 0.5, 2, 5, 10, 25	0, 0.07, 0.28, 0.7, 1.4, 3.5*	- At 2 mg/kg feed, suppressed weight gain and decreased liver and kidney weights.	0.28*	0.07*		119
Rat, Sprague-Dawley, weanling (25 each sex/group)	60 days		0, 0.25, 0.5, 1	- At and above 0.25 mg/kg bw/day in females and at and above 1 mg/kg bw/day in males, decreased weight gain and feed consumption. - At 1 mg/kg bw/day, decreased	0.25			120

Description of Target (Number per group).	Route (solvent) Duration of dosing	Dose (mg/kg feed)	Dose (mg/kg bw/day)	Effects	LOAEL (mg/kg bw/day)	NOAEL (mg/kg bw/day)	Comments	Reference
				thymidine intake rate in the jejunum and spleen.				
Rat, Sprague-Dawley, 190-210 g (10 males /group)	90 days	0,20	0, 1*	- Decreased feed efficiency.	1*			121
Pig, Yorkshire, 10-13 kg (6 neutered males /group)	32 days	0,1, 3	0, 0.08, 0.24*	- At 3 mg/kg feed, decreased feed consumption and weight gain and decreased plasma α-globulin and cortisol.	0.24*	0.08*		122
Pig, Yorkshire, 27.5 kg (3 neutered males /group)	7 weeks	0,4.7	0, 0.19*	- Decreased feed consumption (by 29%) and weight gain (by 27%).	0.19*			106
Pig, 10 kg (9 females /group)	8 weeks	0,0.3, 0.6,1.2	0, 0.012, 0.024, 0.048*	- No decrease in weight gain.		0.048*		123
Pig, 60 kg (3-6 /group)	90 days	0,1	0, 0.04*	- No decrease in weight gain. - No clinical effects. - Kidneys showed such changes as lymphocytic infiltration and tubular epithelium degeneration (not statistically significant).		0.04*		124
Pig, Yorkshire, 12-15-week - old (5 males /group)	2-3 weeks	0, 6 mg/kg DON ＋2 mg/kg 15-AcDON or 3-AcDON		- At 6 mg/kg feed, decreased feed consumption and weight gain. - No major interactive effects were found between DON and other trichothecenes.			No interactive effects between purified DON and 15-AcDON or 3-AcDON.	125
Pig, 9.8 kg (9 females /group)	8 weeks	0,0.3, 0.6,1.2		- No effects on feed consumption and weight gain. - A trend for increased ASAT.				126
Turkey chick, 1-day- old (24 females /group)	21 days	0, 20	0, 1.6*	- No effects on feed consumption, weight gain, hematological and most serological parameters, histological findings or heart and kidney weights. - Decreased serum calcium.	1.6*		Semipurified DON cultured using corn.	127
Rhesus monkey (1-2 /group)	14 days		1, 5	- At and above 1 mg/kg bw/day, decreased platelet count and adhesion capacity as well as decreased fibrinogen levels.	1			128

*: Converted values based on the JECFA standards.

(i) Mouse

In a study lasting for 7 days, BALB/c mice (4 males/group) were fed diets containing 0, 0.25, 5, 10, 20 or 50 mg DON /kg feed (corresponding to 0, 0.35, 0.67, 1.3, 2.7 or 6.5 mg DON /kg bw per day, respectively) all DON-fed groups showed decreased feed consumption. The 10 mg/kg feed and higher dose groups showed decreased body weight and decreased thymus weight. After administration of 10 – 20 mg /kg feed of DON for 2 – 3 weeks, 3 of the 4 mice showed cardiac legions with calcification. The LOAEL and NOAEL were 10 mg/kg feed (i.e., 1.3 mg/kg bw per day) and 5 mg/kg feed (i.e., 0.67 mg/kg bw per day), respectively (115).

After ICR mice (10 each sex/group) were fed diets containing 0, 2, 4 or 8 mg DON /kg feed for 14 days, the 8 mg/kg feed group showed a significant decrease in feed consumption, particularly in males, during both the first and second 7 days. The males in the 2 mg DON /kg feed and higher dose groups showed a decreased weight gain during the initial period, while only the 8 mg/kg feed group showed a decreased weight gain during the second week. The DON-treated groups also showed a significant decrease in red blood cell count (116).

After ICR mice (10 – 12 each sex/group) were fed diets containing 0, 4, 8, 12 or 16 mg DON /kg feed for 14 days, decreased feed consumption was seen at doses above 8 mg/kg. Suppressed body weight gain was observed in all dose groups (117).

Weanling Swiss-Webster mice (24 males/group) received 0, 0.75, 2.5 or 7.5 mg DON/kg bw per day by oral gavage for 35 days. Most mice in the two highest dose groups died during the study period. The 2.5 mg/kg bw per day dose group showed decreased thymocyts and splenic lymphocytes, reduced extramedullary hematopoiesis, gastric mucous gland enlargement, and necrosis of enterocytes lining the cripts in the small intestine. Some effects were observed on the bone marrow (increased reticulocytes and increased erythropoiesis) and hematological parameters (decreased RBC count, hemtocrit, hemoglobin concentration and mean corpuscular hemoglobin concentration [MCHC]). Decreased feed consumption and body weight, decreased relative weights of the thymus and heart, and increased relative stomach weight were seen in all dose groups. The LOAEL was 0.75 mg/kg bw per day (118).

After NMRI mice (10 males/group) were fed diets for 6 weeks containing 0, 0.1, 1 or 10 mg DON/kg feed, weight gain was significantly decreased at a dose of 10 mg/kg (67).

When B6C3F1 mice (8 females/group) were fed diets containing 0, 0.5, 2, 5, 10 or 25 mg DON/kg feed for 56 days, the 2 mg/kg feed and higher dose groups exhibited decreased weight gain. There was a dose-dependent decrease in thymus, spleen, liver, kidney and brain weights, but with no histological changes. The LOAEL and NOAEL were estimated to be 2 and 0.5 mg/kg feed, respectively (i.e., 0.28 and 0.07 mg/kg bw per day, respectively, estimated by JECFA) (119).

(ii) Rat

Sprague-Dawley rats (25 each sex/group) were fed diets containing purified DON at a dose corresponding to 0, 0.25, 0.5 or 1 mg/kg bw per day for 60 days. Decreased body weight gain due to decreased feed consumption was seen in femails of all dose groups and in males in the 1 mg/kg bw per day group. The males in the 1 mg/kg bw per day group also showed a significant decrease in the rate of thymidine uptake by the jejunum and spleen. No significant changes were observed in the hematological and bone marrow parameters, organ weights or histopathological findings. The LOAEL was estimated to be 0.25 mg/kg bw per day in females (120).

After male Sprague-Dawley rats were fed *ad libidum* for 90 days with diets containing 0 or 20 mg purified DON /kg feed, no significant clinical finding was observed. The DON-treated group showd a lower feed efficiency but no feed refusal in spite of a reduced final weight (121).

(iii) Pig

In a subacute toxic studiy, castrated Yorkshire pigs of 10 – 13 kg body weight (6 males/group) were fed diets containing 0, 1 or 3 mg DON /kg added as either purified DON or naturally contaminated corn for 32 days. The estimated amounts of ingested purified DON were 0, 0.08 or 0.24 mg/kg bw per day, respectively, and those of naturally contaminating DON were 0, 0.09 or 0.22 mg/kg bw per day, respectively (estimated by JECFA). The naturally contaminated corn also contained 15-AcDON and NIV at the concentration of 3 and 1.3 mg/kg feed, respectively. A significant decrease in feed consumption and weight gain were seen at dose of 3 mg/kg feed soon after the start of feeding. While weight gain of pigs fed

purified DON recovered in several days, values for pigs fed naturally contaminated DON remained depressed throughout the study period. All DON-treated groups showed lower serum α-globulin levels compared to the control groups. High cortisol levels were observed in the high dose groups (122).

When castrated Yorkshire pigs (9 males/group) were fed diets containing 0 or 4.7 mg purified DON /kg feed for 7 weeks, decreased feed consumption and weight gain were shown in pigs fed DON. The LOAEL was 4.7 mg/kg feed (i.e., 0.19 mg/kg bw per day, as estimated by JECFA) (106).

After pigs (9 females/group) were fed diets containing 0, 0.3, 0.6 or 1.2 mg DON/kg feed for 8 weeks, no significant effects on weight gain were observed. The NOAEL was 1.2 mg/kg feed (i.e., 0.048 mg/kg bw per day, as estimated by JECFA), the highest dose in this study (123).

In a 90-days toxicity study, pigs (3 – 6 pigs/group) were fed diets containing 0 or 1 mg DON/kg feed. The histopathological examination showed a small number of cases with limphyocitic infiltration, tubular epithelium degeneration, etc. in the kidneys caused by 1 mg DON/kg feed, but these were not statistically significant changes (124).

When Yorkshire pigs (5 males/group) were fed diets containing 0 or 6 mg purified DON /kg feed for 2 – 3 weeks, decreased feed consumption and decreased weight gain were observed (125).

In a study in which weanling piglets (9 females/group) were fed diets containing 0, 0.3, 0.6 or 1.2 mg purified DON /kg feed for 8 weeks, no effects on feed consumption or weight gain were observed. Dose-dependent increase of asparagines aminotransferase (ASAT) in blood was observed, but the changes were slight and within the normal range (126).

(iv) Turkey

Turkey chicks were fed diets containing 0 or 20 mg DON /kg for 21 days, starting from day 1 after birth. The consumption of DON caused decrease in serum calcium levels, while there were no effects on feed consumption, body weight gain, the hematological parameters (mean cellular volume [MCV], mean cellular hemoglobin [MCH] and MCH concentration), histological findings or heart and kidney weights (127).

(v) Monkey

A study was conducted in which *Macaca rhesus* monkeys (1 – 2 monkeys/group) were given a single oral dose of 1, 5, 10, 25 or 50 mg DON/kg bw and repeated oral doses of 1 or 5 mg DON/kg bw per day for 2 weeks. One of the 2 monkeys given a single dose of 50 mg/kg bw, dissected at 24 hrs post-dose, showed pleural and epicardial hemorrhage, cerebrovascular swelling, acute enteritis and lymphoid tissue necrosis. Monitoring of the remaining animals over time revealed a trend for decreased blood coagulability starting from 48 hrs post-dose. The decreased blood coagulability continued until 2 weeks post-dose, followed by a trend for recovery to normal coagulability after 1.5 – 2 months. In the repeated-dose study, decreased platlet count and adhesion, decreased fibrinogen levels and other findings suggesting decreased blood coagulability were observed in pigs fed DON at and above 1 mg/kg bw per day. The coagulation parameters restored to normal levels after 1.5 – 2 months (128).

(3) Chronic toxicity and carcinogenicity

In a chronic toxicity study, B6C3F1 mice were fed diets containing DON for 2 years (Table 6). The groups, each consisting of 50 mice of each sex, were fed diets containing 0, 1, 5 or 10 mg DON/kg feed (corresponding to 0, 0.1, 0.5 or 1.1 mg DON/kg bw per day in males and 0, 0.1, 0.7 or 1.6 mg DON/kg bw per day in females; purity of DON >95%; not containing 3-AcDON or 15-AcDON). While the mean daily consumption of feed was not changed in females, a significant decrease (by approximately 8%) in feed consumption was seen in males in the two highest dose groups. Both males and females in the 5 and 10 mg DON/kg feed groups exhibited a significant decrease in body weight. The females in the 5 and 10 mg DON/kg feed groups showed increased serum IgA (by 56%) and IgG (by <10%) concentrations. The males in the 5 and 10 mg DON/kg feed groups showed a decrease in relative weight of liver, while those in the 10 mg/kg feed group showed a decrease in relative weight of spleen and a significant increase in that of testis. Histological analysis of the following organs and tissues showed no increase in the occurrence of preneoplastic or neoplastic lesions in any of them: brain, pituitary, spinal cord, thymus, eyes, lachrymal gland, contiguous harderian gland, nasal turbinates, trachea, lungs, thyroid, adrenal glands, aorta, liver,

spleen, kidneys, pancreas, salivary glands, esophagus, gallbladder, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, lymph nodes, bone marrow, sternum, ureter, prostate, seminal vesicles, testes, mammary gland, uterus, uterine cervix, ovaries, and fallopian tubes, peripheral nerve, and skeletal and smooth muscles. There was a dose-dependent and statistically significant decrease in the occurrence of preneoplastic and neoplastic lesions in the liver, and in the occurrence of nonneoplastic lesions in the islets of Langerhans. The decrease in the occurrence of the proliferative lesions in the liver seemed to reflect the positive correlation between body weight and spontaneous occurrence of hepatocellular carcinoma known in this strain of mice. The NOAEL was 1 mg/kg feed in terms of content in feed (i.e., 0.1 mg/kg bw per day) (129).

Table 6 Long-term toxicity of deoxynivalenol (DON)

Description of Target (Number per group).	Route (solvent) Duration of dosing	Dose		Effects	LOAEL (mg/kg bw/day)	NOAEL (mg/kg bw/day)	Comments	Reference
		(mg/kg feed)	(mg/kg bw /day)					
Mouse, B6C3F1, 22-28-day -old (50 each sex/ group)	Diet, 2 years	0, 1, 5, 10	Males: 0, 0.1, 0.5, 1.1. Females: 0, 0.1, 0.7, 1.6*.	- At and above 5 mg/kg feed, decreased weight gain. – Dose-dependent decrease in the occurrence of tumor.	0.5*	0.1*		129

*: Converted values based on the JECFA standards.

(4) Reproductive/developmental toxicity

Table 7 shows the results of reproductive/developmental toxicity of DON.

(i) Mouse

Developmental toxicity was studied in Swiss Webster mice (7 – 15 males and 10 – 12 females/group) feeding on diets containing 0, 0.375, 0.75, 1.5 or 2.0 mg DON/kg bw per day. After 30 days of dietary feeding, mice (F_0) were mated and gave birth to offspring (F_1a), which were monitored up to 21 days of age. F_0 mice were continually kept to produce litters(F_1b), which were sacrificed at day 19 of gestation. The fetuses (F_1b) were examined macroscopically and checked for visceral and skeletal abnoemalities. Both male and female F_0 mice showed decreased consumption of feed and water at and above 0.375 mg DON/kg bw/day. Female F_0 mice showed decreased body weight at 1.5 mg DON/kg bw/day, with no effects on pregnancy rate. In the 2.0 mg DON/kg bw/day group, the F_1a mice exhibited a decrease in the number of live offspring, the number of postnatal survivors and postnatal body weight, while the F_1b mice exhibited a decrease in the number of live fetuses and mean fetal weight. However, no teratogenicity was observed in the F_1a and F_1b mice (130).

Reproductive toxicity was studied in the following three strains of mice (3 – 6 males/group) feeding on diets containing 0 or 10 mg DON/kg feed for 90 days: IL-6KO [B6129-IL6 (tmlKopf) (IL-6 gene deficient)], WT [B6129F2 (wild type to B6129-IL6 with an intact IL-6 gene)], and B6C3F1 mice. While the DON-fed groups showed a significant decrease in body weight compared to the control group, no histological changes were observed. The DON-fed IL-6KO and B6C3F1 mice showed a significant decrease in cauda epididymis weight (131).

In a study on developmental toxicity, Swiss Webster mice at days 8 – 11 of gestation (15 – 19 females/group) were treated by oral gavage with 0, 0.5, 1, 2.5, 5, 10 or 15 mg DON/kg bw per day. The fetal resorption rate was 100% in the dose groups of 10 and 15 mg DON/kg bw per day, and 80% in the 5 mg/kg bw/day dose group. In the 1, 2.5 and 5 mg DON/kg bw/day dose groups, fetal visceral abnormalities were found at low frequencies. Exencephalia (26%), syndactyly (19%), cerebellar hypoplasia (93%) and other abnormalities were found mainly in the 5 mg/kg bw per day dose group. The 1, 2.5 and 5 mg/kg bw per day dose groups showed skeletal abnormalities in a dose-dependent manner. The NOAEL was 0.5 mg/kg bw/day (132).

Table 7 Reproductive and developmental toxicity of deoxynivalenol (DON)

Description of Target (Number per group).	Route (solvent) Duration of dosing	Dose (mg/kg feed)	Dose (mg/kg bw/day)	Effects	LOAEL (mg/kg bw/day)	NOAEL (mg/kg bw/day)	Comments	Reference
Mouse, Swiss Webster, weanling (7-15 males + 10-20 females/ group)	30-day Diet administra-tion followed by mating		0,0.375, 0.75,1.5, 2	- At 0.375 mg/kg bw/day, decreased feed and water consumption in parent animals. - At 1.5 mg/kg bw/day, decreased maternal body weight. - At 2 mg/kg bw/day, embryotoxicity.	0.375		Reproductive toxicity, 1 generation	130
Mouse, 3 strains (3-6 males /group)	Diet, 90 days	0, 10	0,1.5*	- Suppressed weight gain and decreased cauda epididymis weight.	1.5*		Effects on genitals	131
Mouse, Swiss Webster, 30 g (15-19/ group)	Esophageal intubation (water solution), days 8-11 of gestation		0,0.5,1,2.5, 5,10,15	- At and above 5 mg/kg bw/day, teratogenicity and increased fetal resorption. - At and above 1 mg/kg bw/day, skeletal abnormality.	1	0.5	Developmental toxicity	132
Rat, Sprague-Dawley, male, 325-350 g (12-15/ group)	Oral gavage, 6-19 days		0,0.5, 1.0, 2.5,5.0	- At and above 2.5 mg/kg bw/day, decreased epididymis and seminal vesicle relative weights. - At 5 mg/kg bw/day, decreased prostate relative weight, decreased number of sperm cells and cauda epididymal sperm count, and sperm tail abnormalities.	2.5	1.0	Effects on genitals	131
Rat, Sprague-Dawley, 190-210 g males, 165 g females (10 males +25 females/ group)	Diet, 60 days in males + 15 days in females, both before mating	0, 20	0,2*	- Decreased pregnancy rate.	2*		Reproductive toxicity	132
Rats, Sprague-Dawley, 30-day -old (15 each sex /group)	Diet, 6-week treatment followed by mating, treatment continued during gestation		0,0.25, 0.5, 1	- At 1 mg/kg bw/day, decreased paternal body weight. - At and above 0.25 mg/kg bw/day, pyelectasia and bladder distension in fetuses.	0.25		Reproductive toxicity, 1 generation	130

Description of Target (Number per group).	Route (solvent) Duration of dosing	Dose (mg/kg feed)	Dose (mg/kg bw/day)	Effects	LOAEL (mg/kg bw/day)	NOAEL (mg/kg bw/day)	Comments	Reference
Rat, F344 (23 females/ group)	Diet, 20 days (during gestation)	0, 0.5, 2, 5	0, 0.025, 0.1, 0.25*	- No teratogenicity, no reproductive toxicity. - A trend for decreased maternal body weight (not statistically significant)		0.25*	Developmental toxicity	133
Rat	Oral, days 7-15 of gestation		0, 0.2, 1, 5, 10	- Fetal toxicity. - Delayed ossification.	1	0.2	Developmental toxicity	136
Rat, Sprague-Dawley, female, 201-225 g (24/ group)	Oral gavage, 28 days		0, 0.5, 1.0, 2.5, 5.0	- At and above 1 mg/kg bw/day, mother animals showed dose-dependent decrease in liver weight and histological changes in hepatocytes.	1.0	0.5	Mother animals: Dose-dependent decrease in liver weight was used as an indicator	137
				- At and above 2.5 mg/kg bw/day, fetues showed decreased mean body weight and crown-rump length and decreased ossification of spine.	2.5	1.0	Fetuses: growth inhibition was used as an indicator	
New Zealand White rabbit, 3.2 kg (6-15/ group)	Diet, days 0-30 of gestation	0, 7.5, 15, 30, 60, 120, 240	0, 0.3, 0.6, 1, 1.6, 1.8, 2	- Increased fetal resorption. - Decreased maternal and fetal body weight.	1	0.6	Developmental toxicity	138

*: Converted values based on the JECFA standards.

(ii) Rat

Sprague-Dawley rats (12 – 15 males/group) were given 0, 0.5, 1.0, 2.5 or 5.0 mg purified DON/kg bw per day by oral gavage for 28 days. The 2.5 mg/kg bw per day and higher dose groups showed significant decreases in body weight and in feed consumption as well as in the relative weights of epididymis and seminal vesicle. The 5.0 mg/kg bw per day dose group showed a significant decrease in relative prostate weight, the number of sperm cells, and cauda epididymal sperm count (absolute count and count/g cauda epididymus) as well as a significantly higher occurrence of sperm tail abnormalities (sperm tail damage) than those shown in the control group. All DON-treated groups showed a dose-dependent increase in serum follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels and a dose-dependent decrease in serum testosterone levels. In the histopathological examination, the 2.5 mg purified DON/kg bw per day and higher dose groups showed increased degeneration of reproductive cells and increased sperm retention and abnormal nuclear morphology (133).

A reproductive toxicity study was conducted in premating male (10/group) and female (25/group) Sprague-Dawley rats treatingwith feed containing 0 or 20 mg purified DON/kg feed (corresponding to approximately 2 mg purified DON/kg bw per day, as converted based on the JECFA standards) for 60 and 15 days respectively. The pregnancy rate was 80% in the control group and decreased to 50% in the DON-treated group. There were no differences between the groups in sex ratio or survival rate of the offspring or in the average number or body weight of babies per litter. There were no histopathological changes in testes or ovaries (134).

A reproductive/developmental toxicity was studied in Sprague-Dawley rats (15 each sex/group) treating with feed containing 0.25, 0.5 or 1.0 mg DON/kg bw per day. Females were mated after 6-week treatment with feed containing DON, followed by continuation of the same respective treatments

throughout their gestation period. These females were sacrificed on the last day of pregnancy to examine effects of DON on fetal development. At and above the lowest dose, significant dilatation was observed in the fetal renal pelvis and urinary bladder. No other morphological abnormalities or effects on the number of live fetuses were observed (130).

In another developmental toxicity study, groups of Fischer 344(F344) rats (23 females/group) were treated during their gestation period with feed added with 0, 0.5, 2.0 or 5.0 mg purified DON/kg (corresponding to 0, 0.025, 0.1 or 0.25 mg purified DON/kg bw per day, respectively, as converted based on the JECFA standards). The 2.0 and 5.0 mg/kg feed dose groups showed a trend for lower maternal body weight at the end of pregnancy as well as significantly lower maternal body weight after removal of the uterus and fetuses than in the control group. However, no statistically significant effects on the occurrences of macroscopic, skeletal or visceral abnormalities were observed in any of the treatment groups (135).

After rats were treated on days 7 – 15 of pregnancy by oral gavage with DON solution at a dose of 0, 0.2, 1, 5 or 10 mg DON/kg bw per day, the 1 mg/kg bw/day and higher dose groups showed fetal toxicity (delayed ossification and other skeletal abnormalities). The NOAEL was 0.2 mg/kg bw per day (136).

When Sprague-Dawley rats (24 females/group) were treated on days 6 – 19 of pregnancy by oral gavage with DON solution at a dose of 0, 0.5, 1.0, 2.5 or 5.0 mg DON/kg bw per day, rats of the 5 mg/kg bw per day group showed a significant decrease in maternal feed consumption and body weight. In the same group, 52% of a litter was completely resorbed and the average number of early and late deaths per litter increased significantly. There was also a significant decrease in mean fetal body weight and crown-rump length, a significant increase in the occurrence of prematurity, and a significant decrease in the ossification of fetal sternebrae, vertebral bodies and arches, vertebrae, metatarsi and metacarpi. The 2.5 mg/kg bw per day group showed a significant decrease in mean fetal body weight and crown-rump lenth and vertebral ossification. Relative weight of liver significantly increased in maternal animals of the 1.0 mg/kg bw per day and higher dose groups, suggesting a correlation with the histological changes in hepatocytes. The NOAEL was 0.5 and 1.0 mg/kg bw per day in maternal animals and fetuses, respectively (137).

(iii) Rabbit

In a study to investigate reproductive and developmental toxicity of DON in rabitt, New Zealand White rabbits (6 – 15/group) were fed a diet containing DON at a dose of 0, 0.3, 0.6, 1, 1.6, 1.8 or 2 mg/kg bw per day, on days 0 – 30 of pregnancy. The 1.8 and 2 mg DON/kg bw per day dose groups had a fetal resorption rate of 100%. The 1 and 1.6 mg DON/kg bw/day dose groups showed decreased fetal body weight, which seemed to be caused by decreased maternal body weight and feed consumption. No teratogenicity was found in this study. The NOAEL was 0.6 mg/kg bw per day (138).

(5) Genotoxicity

The results of genotoxicity studies of DON was summarized in Table 8..

In an Ames test using *Salmonella typhimurium*, DON did not induce mutation with or without a metabolic activation using S9(139, 140). An *in vitro* unscheduled DNA synthesis (UDS) test of DON using primary culture of rat liver cells was negative (141). DON did not induce genetic mutation at the *Hprt* locus of V79 cells (142).

In *in* vitro studies, DON induced clastogenic effects in primary culture of rat liver cells (140) and V79 cells (Ref. 143, 144). DON also inhibited gap-junctional intercellular communication (145).

DON promoted the transformation of mouse BALB/3T3 cells (146). In a short-term transformation assay system using v-Ha-ras transfected BALB/3T3 cells, however, DON showed no initiation or promotion activity (147).

In a comet assay using splenic WBCs collected from 10 male broilers that had ingested 10 mg DON/kg feed for 17 days, DON induced minor but significant DNA damage (148).

Table 8 Results of genotoxicity studies of deoxynivalenol (DON)

Table 8-1: *in vitro*

End-point	Test object	Concentrations	Results	Reference
Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537*	0.4 - 400 µg/plate	Negative	139
Reverse mutation	*S typhimurium* TA98, TA100 *	0.7 - 500 µg/plate	Negative	140
Reverse mutation	SOS system using *E. coli* PQ37 *	5 - 500 µg/assay	Negative	140
Gene mutation	*Hprt* gene of Chinese hamster V79 cells**	1 - 3 µg/mL ***	Negative	142
Unscheduled DNA synthesis	Primary culture of rat liver cells	0.1 - 1,000 µg/mL	Negative	141
DNA repair	*E. coli* K12 (2 strains)	0.7 - 500 µg/mL	Negative	140
Chromosome aberrations	Chinese hamster V79 cells	0.1 - 1 µg/mL	Positiv. (5-fold)	143
Chromosome aberrations	Chinese hamster V79 cells	0.03 - 0.3 µg/mL	Positive (5-fold)	144
Chromosome aberrations	Primary rat liver cell cultures	0.001 - 100 µg/mL	Positiv. (6-fold)	140
Micronucleus formation	Primary culture of rat liver cells	Up to 100 µg/mL	Negative	140
Gap-junctional intercellular communication	Chinese hamster V79 cells	0.1 - 0.5 µg/mL	Inhibited	145
Transformation	BALB/3T3 mouse embryo cells	0.1 - 1.6 µg/mL	Positive	146
Transformation	v-Ha-ras transfected BALB/3T3 mouse embryo cells	0.01 - 0.2 µg/mL	Negative	147

*: With or without activation with S9.
**: With or without metabolic activation using liver cells.
***: At 1 µg/mL, reduced colony size; at 10 µg/mL, a cell death rate of 90%.

Table 8-2: *in vivo*

End-point	Test object	Results	Reference
DNA damage (comet assay)	Splenic WBCs of male broilers fed DON (10 mg/kg feed) for 17 days	Positive	148

(6) Other toxicities (immunotoxicity, hematotoxicity, etc.)

(i) Immunotoxicity

a. Effects on immune responseson and on resistance to infection

Table 9 provides a summary of the effects of DON on immune responses and resistance to infection. Esposure to DON has been reported to be associated with decreased thymus and spleen weights, decreased resistance to infection, and leucopenia as descrived below.

(a) Mouse

Immunotoxicity of DON was studied in Swiss Webster mice (weanling; 12 males/group) giving DON at a dose of 0, 0.75, 2.5 or 7.5 mg/kg bw per day by oral gavage for 5 weeks. All mice in the 7.5 mg/kg bw per day dose group died within 3 weeks. The 0.75 and 2.5 mg/kg bw per day dose groups showed suppressed antibody responses to sheep RBCs as well as decreased thymus weight. The LOAEL was 0.75 mg/kg bw

per day (149).

In an additional immunotoxicity study by the same research group, Swiss Webster mice (6 – 10 males/group) were orally given purified DON at a dose of 0, 0.25, 0.5 or 1 mg purified DON/kg bw per day. Mice in the 0.5 mg/kg bw per day or higher dose groups showed a significant decrease in serum levels of α2-globulin and β-globulin, and a dose-related reduction in time-to-death interval following the challenge with *Listeria monocytogenes* (*L. monocytogenes*)to death. The NOAEL was 0.25 mg purified DON/kg bw per day (150).

The immunosuppressive effects of DON were confirmed in a study, in which B6C3F1 mice (8 – 11 females/group) were fed a diet containing 0, 5 or 25 mg purified DON/kg feed for 2 – 3 weeks. DON reduced plaque- forming cell response to sheep red blood cells and delayed hypersensitivity response to keyhole limpet hemocyanin, as well as decreased ability to resist *L. monocytogenes* in mice in the 25 mg/kg feed dose group as compared to those in the control group. At 5 mg/kg feed (corresponding to 1 mg/kg bw per day, as estimated by JECFA), none of the above parameters was affected. The NOAEL was 5 mg/kg feed (i.e., 1 mg/kg bw per day) (151).

In a subclonic stucy, B6C3F1 mice (8 females/group) were fed diets containing purified DON at a concentration of 0, 0.5, 2, 5, 10 or 25 mg/kg feed (corresponding to 0, 0.1, 0.4, 1, 2 or 5 mg purified DON/kg bw per day, respectively, as estimated by JECFA) for 8 weeks. A dose-dependent decrease in the white blood cell count was observed at doses of10 mg/kg feed and above. The NOAEL was 5 mg/kg feed (i.e., 1 mg/kg bw per day) (119).

In an immunotoxicity study, BALB/c mice (4 - 17 males/group) were fed diets containing DON at aconcentration of 0, 2.5, 5, 10, 20 or 50 mg/kg feed (corresponding to 0, 0.37, 0.75, 1.5, 3 or 7.5 mg DON/kg bw per day, respectively, as estimated by JECFA) for 1 – 2 weeks. Mice in the 10 mg /kg feed and higher dose groups showed decreased thymus weight with atrophy, as well as a significant decrease in: response to sheep RBCs; splenic lymphocyte response to phytohemagglutinin (PHA) and lypopolysaccharides; and thymal lymphocyte response to PHA. The NOAEL was 5 mg/kg feed (i.e., 0.75 mg/kg bw per day) (152).

The effects of DON on host resistance to i *Salmonella enteritidis* (*S. enteritidis*) was studied by treating BALB/c mice (10 males/group) with drinking water containing DON at a concentration of 0, 0.2, 1 or 3 mg/L (corresponding to 0, 0.024, 0.12 or 0.36 mg DON/kg bw per day, respectively) for 4 weeks. After intragastric administration of *S. enteritidis* on day 14 of treatment, both 1 and 3 mg/L dose groups exhibited a decreased survival rate due to infection, while the 0.2 mg/L dose group showed no change in survival rate. In an analysis of immune reponse to *S. enteritidis* in mice exposed to DON at a concentration of 2 mg/L for 3 weeks, the animals exhibited decreased resistance to the bacterial infection. A significant reduction was also seen in serum anti-*S. enteritidis* IgM and in delayed-type hypersensitivity reactions. The LOAEL was 1 mg/L (i.e., 0.12 mg/kg bw per day) (153).

In another study, BALB/c mice (10 females/group) were treated with drinking water containing DON at a concentration of 0, 0.2, 2 or 6 mg/kg for 4 weeks, following infection with *S. enteritidis* on day 14 of DON-treatment. DON decreased survival of salmonellosis and increased serum TNF-α levels in mice in the 2 mg/kg or higher dose groups. Serum TNF-α levels were decreased in mice at 0.2 mg DON/kg (154).

In an immunotoxicity study, BALB/c mice (6 females/group) recieved a single dose of 0, 2, 5, 10 or 25 mg DON/kg bw by with a gavage, followed by intranasal infection with reovirus at 2 hrs post-treatment. After 3 days, the DON-treated groups showed a larger number of reovirus L_2 RNA copies as well as decreased mRNA expression for interferon (INF)-α, the IFN-αβ receptor and IFN-γ receptor in lung, compared to non-treatment group. In addition, increased MCP-1 and TNF-α production, accumulation of inflammatory cells, and increased reovirus-specific IgA were observed in bronchoalveolar lavage fluid in mice exposed to DON (155).

When BALB/c mice (4 males/group) were fed diets containing DON at a concentration of 0, 2.5, 5, 10, 20 or 50 mg/kg feed (corresponding to 0, 0.35, 0.67, 1.3, 2.7 or 6.5 mg DON/kg bw per day, respectively) for 1 week, animals in the 10 mg/kg feed and higher dose groups showed a significant decrease in thymus weight. The NOAEL based on decreased thymus weight was 5 mg/kg feed (i.e., 0.67 mg/kg bw per day) (115).

BALB/c mice (12 males/group) were fed diets containing DON at a concentration of 0 or 2 mg/kg feed (corresponding to 0.3 mg DON/kg bw per day[5])for 14 days followed by a running exercise on a treadmill

[5] The intake was estimated using the relevant conversion factors used by JECFA (IPCS: EHC70).

until exhaustion. Significant inhibition of splenocyt proliferation in response to concanavalin-A stimulation *in vitro* was observed when mice had been given DON without exercise (156).

Lactating inbred Han:NMRI mice (5 – 10/group) recieved a single gavage containing 0 or 12.5 mg DON/kg bw, or treated with 6.25 mg DON/kg bw per day for 7 consecutive days. DON was found to alleviate the morbidity caused by infection with mastitis-causing bacteria *Staphylococcus hyicus* and *Mycrobacterium avium*. It was suggested that the increased serum IgA, IgM and IgG levels were involved in this effect (157).

(b) Chicken

In an immunotoxicity study, groups of ten 1-day old layer chicks (White Leghorn) were fed diets containing wheat uncontaminated or naturally contaminated with DON at a concentration of 18 mg/kg feed (corresponding to 2.25 mgDON/kg bw per day) for 18 weeks, DON caused suppression of antibody responses to a Newcastle disease vaccine. When 1-day old broilers in groups of 3 were given a single dose of feed containing 0 or 50 mg DON/kg (corresponding to 6.25 mg DON/kg bw per day, as estimated by JECFA), DON-contaminated diet caused a suppression of lymphocyte blastogenesis (158).

(c) Pig

When Norwegian Landrace pigs (8 each sex/group) were fed with naturally contaminated oats containing 0.6, 1.8 or 4.7 mg DON/kg (corresponding to 0.024, 0.072 or 0.2 mg DON/kg bw per day, respectively, as estimated by JECFA) for 9 weeks, a dose-dependent decrease in the secondary antibody response to tetanus toxin was shown in aminals (159).

When pigs (7 males/group) were given DON in diets at a dose of 0 or 0.5 mg DON/kg bw per day for 1 week followed by an additional treatment with DON at a dose of 1 mg/kg bw per day for 5 weeks, DON caused no histopathological changes in lymphocyte subsets, hematological parameters or lymphoid tissue (160).

In an immunotoxicity study,pigs (6 neutered males or females/group) were fed diets contaminated with DON at a concentration of 0, 0.28, 0.56 or 0.84 mg/kg feed for 28 days. No changes were found in hematologyical variables (white blood cell, red blood cell and platelet counts, relative number of neutrophils and lymphocytes, hematocrit and hemoglobin levels, etc.) or blood biochemistry values (cations, glucose, urea, creatinine, bilirubin, cholesterol and triglyceride levels, plasma enzyme activities, etc.). No effects on immune responses (immunoglobulin subset concentration, lymphocyte proliferation, and cytokine production) were observed (161).

Species	Final body weight (kg)	Intake (g/animal/day)	Intake (g/kg bw/day)
Mouse	0.02	3	150

Table 9 Immunological toxicity of orally administrated deoxynivalenol (DON)

Description of Target (Number per group).	Route (solvent) Duration of dosing	Dose (mg/kg feed)	Dose (mg/kg bw/day)	Effects	Minimum immunotoxic dose (mg/kg bw/day)	Maximum non-immunotoxic dose (mg/kg bw/day)	Comments	Refefence
Mouse, Swiss Webster, weanling (12 males/ group)	Oral gavage (solvent: propylene glycol, ethanol, distilled water), 5 weeks		0, 0.75, 2.5, 7.5	- Deaths at 7.5 mg/kg bw/day - At 0.75 and 2.5 mg/kg bw/day, suppression of antibody responses to sheep RBCs and decreased thymus weight.	0.75		Antibody responses	149
Mouse, Swiss Webster, 21-day old (6-10 males/ group)	Diet, 5 weeks		0, 0.25, 0.50, 1	- At and above 0.50 mg/kg bw/day, decreased serum α2-globulin and β-globulin levels and reduced time between infection with *L. monocytogenes* and death.	0.5	0.25	Host resistance	150
Mouse, B6C3F1, 15-18 g (8-11 females/ group)	Diet, 2-3 weeks	0, 5, 25	0, 1, 5*	- At 25 mg/kg feed, decreased plaque forming cell response to sheep RBCs, delayed hypersensitivity reactions, and decreased resistance to infection with *L. monocytogenes*.	5*	1*	Antibody responses, hyper-sensitivity reactions, host resistance	151
Mouse, B6C3F1 (8 females/ group)	Diet, 8 weeks	0, 0.5, 2.0, 5.0, 10, 25	0, 0.1, 0.4, 1, 2, 5*	- At and above 10 mg/kg feed, decreased WBC count.	2*	1*		119
Mouse, BALB/c, 4-6-week old (4-17 males/ group)	Diet, 1-2 weeks	0, 2.5, 5, 10, 20, 50	0, 0.37, 0.75, 1.5, 3, 7.5*	- At and above 10 mg/kg feed, decreased responses to sheep RBCs, decreased splenic and thymal leukocyte responses to mitogens, and decreased thymus weight.	1.5*	0.75*	Antibody responses	152
Mouse, BALB/c, 7-week old (10 males/ group)	Potable water, 4 weeks	0, 0.2, 1, 3 mg/L	0, 0.024, 0.12, 0.36	- At 1 and 3 mg/L, decreased survival rate due to *S. enteritidis* infection.	0.12	0.024	Host resistance	153
Mouse, BALB/c, 7-week old (10 females/ group)	Potable water, 4 weeks	0, 0.2, 2, 6		- At and above 2 mg/kg, decreased survival rate due to *S. enteritidis* infection and increased TNF-α production.			Host resistance	154
Mouse, BALB/c, 5-week old (6 females/ group)	Single oral gavage (solvent: water)		0, 2, 5, 10, 25	- At and above 2 mg/kg bw, severer reovirus infection.	2		Host resistance	155

Description of Target (Number per group).	Route (solvent) Duration of dosing	Dose (mg/kg feed)	Dose (mg/kg bw/day)	Effects	Minimum immunotoxic dose (mg/kg bw/day)	Maximum non-immunotoxic dose (mg/kg bw/day)	Comments	Refefence
Mouse, BALB/c, 4-6-week old (4 males/ group	Diet, 7 days	0, 2.5, 5, 10, 20, 50	0, 0.35, 0.67, 1.3, 2.7, 6.5	- At and above 10 mg/kg feed, decreased thymus weight.	1.3	0.67		115
Mouse, BALB/c, 8-week old (12 males/ group)	Diet, 14 days	0, 2	0, 0.3**	- Suppression of splenocyte proliferation.	0.3**			156
Mouse, Han:NMRI, 8-10-week old (5-10/ group)	Oral gavage (solvent: 2% ethanol), 1 week		0, 6.25	- Increased resistance to *S. hyicus* and *M. avium*, and increased serum IgA, IgM and IgG levels.			Host resistance	157
Chicken, broiler (10 females/ group)	Single Diet dose (naturally contaminated feed)	0, 50	0, 6.25*	- Suppression of splenic lymphocyte blastogenesis to PHA.	6.25*			158
Pig, Norwegian Landrace, 25.3 kg (8 each sex/group)	Diet, 9 weeks (naturally contaminated feed)	0.6, 1.8, 4.7	0.024, 0.072, 0.2*	- Dose-dependent decrease in the secondary antibody response to tetanus toxin (no control group fed a diet without toxin).			Host resistance	159
Pig, 8-weekold (7 males/ group)	Oral, 6 weeks		0 or 0.5 for the 1st week followed by 0 or 1 for the next 5 weeks	- No histopathological changes in hematological parameters or lymphoid tissue.				160
Pig, 11.2 kg (6 each sex/group)	Diet, 28 days (naturally contaminated feed)	0, 0.28, 0.56, 0.84		- No effects on immune responses.				161

*: Estimated by JECFA.
**: The intake was estimated using the relevant conversion factors.

b. Changes in serum IgA levels and IgA nephropathy

The effects of DON on IgA have been studied in experimental and other animals. In mice, nephropathy associated with IgA accumulation in renal glomerular mesangial cells have been reported (Table 10).

In a study in which B6C3F1 mice (8 females/group) were fed diets containing purified DON at a concentration of 0, 0.5, 2, 5, 10 or 25 mg/kg (corresponding to 0, 0.1, 0.4, 1, 2 or 5 mg/kg bw per day, respectively, as estimated by JECFA) for 6 weeks, increased serum IgA levels were observed in groups at doses of 2, 5 and 10 mg purified DON/kg feed, while the serum IgM level was decreased in animals in the 25 mg/kg feed dose group. The NOAEL was 0.5 mg/kg feed (i.e., 0.1 mg purified DON/kg bw per day) (119).

When B6C3F1 mice (6 – 13 females/group) were fed diets containing purified DON at a concentration of 0, 2, 10, 25 or 50 mg/kg for 24 weeks, the most significant increase in serum IgA level was seen in mice of the 25 mg/kg dose group (corresponding to 5 mg purified DON/kg bw per day, as estimated by JECFA). The level of IgA in those mice that peaked at 24 weeks was 17-fold of that of the control group. Concurrently, serum IgM and IgG levels were decreased. In mice of the 25 mg/kg dose group a significant increase in IgA production in splenocytes as well as IgA accumulation in renal glomerular mesangium were

observed (162).

To examine the effects on serum IgA production, B6C3F1 mice (7 – 9 each sex/group) were fed diets containing 0, 2, 10 or 25 mg/kg of DON (corresponding to 0, 0.4, 2 or 5 mg DON/kg bw per day, respectively, as estimated by JECFA) for 12 weeks. The increase of the serum IgA levels were seen in males in the 10 mg/kg feed and higher dose groups and females in the 25 mg/kg feed dose group at week 4. At week 8, increased serum IgA levels were also observed in males in the lowest 2 mg/kg feed dose group and females in the 10 mg/kg feed dose group. At week 12, however, a significant increase in serum IgA was seen only in the 10 mg/kg feed dose group. Dose-dependent increase in IgA accumulation in renal glomerular mesangial cells was observed in males with intensity higher than in females. Microscopic hematuria was observed in males in all DON-treated groups from week 4 onward and in females in the 10 mg/kg feed and higher dose groups at week 12 (163).

In a 2-year study, B6C3F1 mice (50 each sex/group) were fed diets containing purified DON at a concentration of 0, 1, 5 or 10 mg/kg feed (corresponding respectively to 0, 0.1, 0.5 or 1.1 mg purified DON/kg bw per day, respectively, in males, and 0, 0.1, 0.7 or 1.6 mg/kg bw per day, respectively, in females, as estimated by JECFA). Females in the 10 mg/kg feed dose group showed a significant increase in serum IgA (129).

In another study, B6C3F1 mice (5 – 6 females/group) were fed diets containing purified DON at a concentration of 0 or 25 mg/kg (corresponding to 0 or 5 mg/kg bw per day, respectively, as estimated by JECFA) for 4, 8 or 12 weeks. A time-dependent increase in serum IgA was shown in the DON-treated group after 4 weeks. A significant increase in IgA production in Peyer's patch lymphocytes and splenic lymphocytes were observed (164, 165).

When B6C3F1 mice (9 females/group) were fed diets containing purified DON at concentration of 0 or 25 mg/kg feed (corresponding to 5 mg/kg bw per day, as estimated by JECFA) for 8 weeks. Increased serum IgA, as well as a significant increase in IgA production in Peyer's patch lymphocytes and splenic lymphocytes, was observed in mice given diets containing DON (166).

B6C3F1 mice (4 males/group) that were given a single oral exposure of purified DON at a dose of 0, 5 or 25 mg /kg bw/day by gavage. A significant increase in IgA production in Peyer's patch lymphocytes was observed at 2 hrs after oral exposure to DON. The increased IgA production was also seen at 24 hrs after oral exposure to DON (167).

In C57BL/6 mice (10 males/group), which received 0, 0.071 or 0.355 mg DON /kg bw (solvent: 5% gum arabic water solution) 3 times a week for 4 weeks by gavage, either alone or in combination with NIV, plasma IgA increased following exposure to the toxins. The liver ethoxyresorufin *O*-dealkylase, pentoxyresorufin *O*-deepenthylase and GST activities were increased together with the expression of CYP1a and CYP2b subfamilies (168).

In an immunotoxicity study, B6C3F1 mice (6 males/group) were orally exposed to DON at doses of 0, 0.83, 2.5 or 7.5 mg /kg bw per day for 8 consecutive days. At 7.5 mg DON/kg bw per day, decreased plasma IgA levels but no change in IgE levels were observed. At concentrations above 2.5 mg/kg bw per day, increased haptoglobin were seen. There was a dose-dependent decrease in IgG and IgM at doses above 0.83 mg/kg bw per day. The LOAEL was 0.83 mg DON/kg bw per day (169).

IgA nephropathy in B6C3F1 mice (12 females/group) was studied by feeding diets containing DON at a concentration of 0 or 25 mg/kg (corresponding to 0 or 5 mg/kg bw per day, respectively) for 24 weeks. The exposure to DON increased serum IgA levels in mice, resulting in marked IgA in mice accumulation in glomerular mesangial cells, similar to that seen in human glomerulonephritis. The IgA accumulation in the kidneys persisted for at least 16 weeks after withdrawal period following the 8weeks dietary exposure to DON (170).

The effects of DON was compaired in B6C3F1 mice (8 – 9 females/group) fed diets containing purified DON at a concentration of 0 or 20 mg/kg, either continuously or intermittently at intervals of 1 week, for 13 weeks. Body weight of animals in the continuous exposure group was consistently lower than that of control, while body weight of animals in the intermittent exposer group was also lower but with a tendency to increase during the non-exposed periods. Serum IgA levels in the group of intermittent exposure remained at control levels, and higher serum IgA levels were observed in the continuous exposure group. Serum IgG and IgM levels were decreased in both of the intermittent and the continuous exposure groups compared with the control group. The IgA accumulation in renal mesangial cells in the intermittent group was less than the accumulation in the continuous group, and was comparable to the control group (171).

In order to investigate the potential involvement of IL-6 in IgA production and accumulation in renal mesangial cells, highly sensitive B6C3F1 mice (3 males/group) and IL-6 knockout mice (B6126-IL6$^{tmi\ Kopf}$) and their wild-type counterparts (B6120F2, 6 males/group) were given diets containing DON at a concentration of 0 or 10 mg/kg for 12 weeks. Dietary exposure to DON decreased feed consumption and body weight in mice compared to those in the non-DON-exposed mice. The exposure to DON resulted in a significant increase in serum IgA and IgA accumulation in renal mesangial cells in B6C3F1 and wild-type mice, while no increase in serum IgA and evidently less IgA accumulation in renal mesangial cells were observed in IL-6 knockout mice (172).

In order to further investigate the potential involvement of COX-2 in IgA production, the same researchers fed diets containing DON at a concentration of 0 or 10 mg/kg to B6C3F1 mice, COX-2 knockout mice (B6, 129P2-*Ptgs2*^{tmlsmi} (002181-M; COX-2-knockout)) and their wild-type counterparts (B6, 129P2-*Ptgs2*^{tmlsmi} (002181-W)) for 16 weeks. In COX-2 knockout mice as well as in their wild-type counterparts, the exposure of DON resulted in increased serum IgA, accumulation of IgA immune complexes (ICs), IgA accumulation in the kidneys, and increased splenic IgA secretion. The DON-induced increase in serum IgA was enhanced in COX-2 knockout mice. Similar results were obtained in a study using COX-2 inhibitors, where suppression of COX-2 activity resulted in enhancement of the DON-induced increase in serum IgA (173).

The effects of DON on IgA production have been studied in systemic lupus erythematosus[6] mouse models (3 strains: NZBW/F_1, MRL/lpr and BXSB), fed diets containing DON at a concentration of 0, 5 or 10 mg/kg (corresponding to 0, 0.75 or 1.5 mg/kg bw per day[7], respectively) for 9 – 14 weeks. No changes were observed in their serum IgA levels, while BXSB mice in the 10 mg/kg dose group had increased IgA accumulation in renal mesangial cells. None of these immunologically abnormal mouse strains seemed to be more sensitive to DON than other common inbred mouse strains (174).

When Wistar rats (6 males/group) were orally given DON at the dose of 0 or 7.5 mg/kg bw for 8 consecutive days, increased haptoglobin and decreased IgG and IgA levels were observed in the DON exposed animals (169).

In a study in pigs (9 – 10/group), the animals were given feed naturally contaminated with 2.2 – 2.5 mg DON/kg or non-contaminated feed for 9 weeks. No trichothecenes other than DON were detected in the feed. At days 4 and 15 after the start of the exposure, the animals were subcutaneously inoculated with ovalbumin (OVA). In animals fed the contaminated feed, serum IgA and OVA-specific IgA and IgG levels were increased. In those animals, decreased levels of mRNA expression of TNF-α and IFN-γ in the mesenteric lymphoid tissue were observed. There were no effects on hematologic or biochemical parameters (175).

When pigs (8 – 9 females/group) were fed diets containing purified DON at a concentration of 0, 0.3, 0.6 or 1.2 mg/kg for 8 weeks, the dose of the 0.6 mg/kg feed and higher resulted an increase in serum IgA levels (176). While, in Norwegian Landrace pigs (7 – 11 each of females and neutered males/group) treated with naturally-contaminated oats containing 0, 0.7, 1.7 or 3.5 mg DON/kg feed (corresponding to 0, 0.04, 0.1 or 0.2 mg DON/kg bw per day, respectively, as estimated by JECFA), no changes in serum IgA levels were observed (177).

[6] Systemic lupus erythematosus (SLE) is an autoimmune disease of unknown cause involving inflammation in many organs throughout the body.

[7] The intake was estimated using the relevant conversion factors used by JECFA (IPCS: EHC70).

Species	Final body weight (kg)	Intake (g/animal/day)	Intake (g/kg bw/day)
Mouse	0.02	3	150

Table 10 Effects of deoxynivalenol (DON) on IgA levels in maouse, rat and pig

Description of Target (Number per group).	Route (solvent) Duration of dosing	Dose (mg/kg feed)	Dose (mg/kg bw/day)	Effects	Minimum dose affecting IgA production (mg/kg bw/day)	Maximum dose not affecting IgA production (mg/kg bw/day)	Refefence
Mouse, weanling, B6C3F1 (8 females/ group)	Diet, 6 weeks	0, 0.5, 2.0, 5.0, 10, 25	0, 0.1, 0.4, 1, 2, 5*	- At and above 2.0 mg/kg feed, increased serum IgA. - At 25 mg/kg feed, decreased serum IgM levels.	0.4*	0.1*	119
Mouse, B6C3F1, 8-10-week-old (6-13 females/ group)	Diet, 24 weeks	0, 2, 10, 25, 50	0, 0.4, 2, 5, 10*	- At 25 mg/kg feed of DON, maximally elevated serum IgA levels, decreased IgG and IgM, and increased IgA accumulation in renal glomerular mesangium.			162
Mouse, B6C3F1, 8-week -old (7-9 each sex /group)	Diet, 12 weeks	0, 2, 10, 25	0, 0.4, 2, 5*	- At 10 mg/kg feed, persistent increse in serum IgA. A dose-dependent increase in IgA accumulation in mesangial cells (particularly in males).	2*	0.4*	163
Mouse, B6C3F1 (50 each sex/ group)	Diet, 2 years	0, 1, 5, 10	Males: 0, 0.1, 0.5, 1.1*; Females: 0, 0.1, 0.7, 1.6*	- At 10 mg/kg feed, a significant increase in serum IgA in females.	1.6*	0.7*	129
Mouse, B6C3F1, 8-10-week-old (5-6 females/ group)	Diet, 4, 8, 12 weeks	0, 25	0, 3.75**	- A time-dependent increase in serum IgA, and a significant increase in IgA production in Peyer's patch lymphocytes and splenic lymphocytes.	3.75**		164 165
Mouse, B6C3F1, 8-10-week-old (9 females/ group)	Diet, 8 weeks	0, 25	0, 3.75**	- Increased serum IgA and a significant increase in IgA production in Peyer's patch lymphocytes and splenic lymphocytes.	3.75**		166
Mouse, B6C3F1, 8-9-week-old (4 males/ group)	Single oral gavage (carbonate buffer)		0, 5, 25	- At and above 5 mg/kg bw/day, increased IgA production detected in the Peyer's patch cell culture medium.	5		167
Mouse, C57BL/6, 6-week-old (10 males/ group)	Oral gavage (5% gum arabic water solution) 3 days /week for 4 weeks		3 doses/ week at 0, 0.071, 0.355 mg/kg bw	- Increased plasma IgA.	0.03***		168
Mouse, B6C3F1, 8-week-old (6 males/ group)	Oral gavage (water solution), once daily for 8 days		0, 0.83, 2.5, 7.5	- A dose-dependent decrease in serum IgG and IgM. - At 7.5 mg/kg bw DON, decreased IgA levels. - No changes in IgE levels.	7.5	2.5	169

Description of Target (Number per group).	Route (solvent) Duration of dosing	Dose		Effects	Minimum dose affecting IgA production (mg/kg bw/day)	Maximum dose not affecting IgA production (mg/kg bw/day)	Refefence
		(mg/kg feed)	(mg/kg bw/day)				
Mouse, B6C3F1, 8-9-week-old (12 females/ group)	Diet, 24 weeks	0, 25	0, 3.75**	- Increased serum IgA and IgA accumulation in renal mesangial cells.	3.75**		170
Mouse, B6C3F1, 7-8-week-old (8-9 females/ group)	Diet, 13 weeks	0, 20	0, 3**	- Increased serum IgA and IgA accumulation in renal mesangial cells.	3**		171
Mouse, B6C3F1, B6129F2 and IL-6 knockout mouse, 4-week-old (3-6 males/ group)	Diet, 12 weeks	0, 10		- Decreased feed consumption and body weight in all DON-treated groups compared with the non-treatment groups. - Of the DON-treated groups, IL-6KO mice showed less increase in blood IgA levels and less IgA accumulation in renal mesangial cells.			172
Mouse, B6C3F1, B6129F2 and COX-2 knockout mouse, 7-8-week-old (5-6 females/ group)	Diet, 16 weeks	0, 10, 25		- In the wild-type mice, DON induced increased serum IgA, accumulation of IgA immune complexes (ICs), IgA accumulation in kidneys, and splenic IgA secretion. - COX-2 knockout mice showed an enhanced increase in serum IgA. - COX-2 inhibitors enhanced DON-induced increase in serum IgA.			173
Mouse, female NZBW/F1, female MRL /lpr, male BXSB, 5-6-week-old (7/group)	Diet, 9-14 weeks	0, 5, 10	0, 0.75, 1.5**	- No changes in serum IgA levels. - Only in BXSB mice in the 10 mg/kg feed group, increased IgA accumulation in renal mesangial cells.			174
Rat, Wistar, 8-week-old (6 males/ group)	Oral (water solution), 8 days		0, 7.5	- Decreased serum IgG and IgA.	7.5		169
Pig (9-10 /group)	Diet, naturally-contaminated wheat (no trichothecenes other than DON detected), 9 weeks	2.2 -2.5		(At days 4 and 15, subcutaneous inoculation with ovalbumin (OVA).) - In the DON-treated group, increased serum IgA and OVA-specific IgA, and decreased mRNA expression of TNF-α and IFN-γ in the mesenteric lymphoid tissue.			175

Description of Target (Number per group).	Route (solvent) Duration of dosing	Dose (mg/kg feed)	Dose (mg/kg bw/day)	Effects	Minimum dose affecting IgA production (mg/kg bw/day)	Maximum dose not affecting IgA production (mg/kg bw/day)	Refefence
Pigs, 9.8 kg (8-9 females /group)	Diet, 56 days	0, 0.3, 0.6, 1.2		- At and above 0.6 mg/kg feed, a trend for increased serum IgA levels.			176
Pigs, females and neutered males, 59-day old, 21.3 kg (7-11 each sex/ group)	Diet, 96 days	0, 0.7, 1.7, 3.5 (naturally-contaminated oats)	0, 0.04, 0.1, 0.2	- No changes in serum IgA		0.2	177

*: Estimated values using the relevant conversion factors set by JECFA.
**: The intake was estimated using the relevant conversion factors.
***: The 3-times-weekly doses were converted to daily doses.

c. Cytokine expression

It has been reported that DON induces interleukins and other inflammatory and immunological cytokines by modulating gene expression level.

When B6C3F1 mice (5 males/group) were treated orally with 0 or 25 mg DON/kg bw following 2 hrs of fasting and were analyzed for changes in gene expressions in the spleen at 2 hrs post-dose by microarray analysis, DON increased expressions of genes associated with immunity, inflammation and chemotaxis, including IL-1α, IL-1β, IL-6, IL-11 and macrophage inhibitory protein-2 (MIP-2) (178).

When IL-2 production in a mouse-T-cell lin was studied *in vitro*, increased transcriptional activity involving the intracellular signal transducers NF-$_K$B and AP-1 was observed in the presence of DON at concentrations of 100~250 ng/mL (179, 180). DON increased the stability of IL-2 mRNA in this T-cell line (181). In U937 cells (a human leukemia-derived cell line), p65 a NF-$_K$B subunit seemed to be involved in DON-increased IL-8 expression at a concentration of 1 μg/mL of DON (182).

Effects of DON on the cytokine mRNA expression in the spleen and Peyer's patches were studied in B6C3F1 mice (3 females/group) giving a single gavage containing purified DON at a dose of 0, 0.1, 0.5, 1, 5 or 25 mg/kg bw, and analyzing the effects at 2 hrs post-dose. Treatment with 5 or 25 mg DON/kg bw significantly induced mRNA expression of: the inflammatory cytokines IL-1β, IL-6 and TNF-α; the type 1 T helper (Th1) cytokines IFN-γ and IL-2; and the type 2 T helper (Th2) cytokines IL4 and IL10. IL-12p40 mRNA was also induced, while IL-12 p35 mRNA was not. These effects were more prominent in the spleen than in Peyer's patches. The NOAEL was 1 mg/kg bw per day (183).

To examin effects of purified DON, B6C3F1 mice (3 males/group) were given per os 0, 0.5, 2 or 5 mg purified DON/kg bw per day for 2, 4 or 7 days and the effects on the cytokine mRNA expression in the spleen and Peyer's patches were examined 2 hrs after the last treatment. A dose-dependent increase in mRNA expression of IL-1β, IL-6, TNF-α, IL-12p35, IL-12p40, IL-2 and IL-10 was observed, while there were no effects on mRNA expression of IFN-γ or IL-4. The NOAEL was 0.5 mg/kg bw per day (184).

In C57BL/6 mice (3 females/group), effects of DON orally given at 0, 1, 5 or 25 mg/kg bw once with gavage were examined. In the 25 mg/kg bw dose group, the COX-2 mRNA expression in Peyer's patches and the spleen peaked 2 hrs after exposure. The IL-6 mRNA expression peaked from 2 to 4 hrs after exposure (185).

B6C3F1 mice (15 males/group) were treated with gavage at doses of 0 or 25 mg DON/kg bw and analyzed for the effects on cytokine mRNA expression. At 2 hrs post-dose, induction of cytokines (IL-1α, IL-1β, IL-6 and IL-11), chemokines (MCP-1, MCP-3, CINC-1 and MIP-2), components of the AP-1 complex (c-Fos, Fra-2, c-Jun and JunB), and two dephosphorylating enzymes (MKP1 and CnAβ) were observed in spleen of DON exposed mice. The induction of mRNA expression was transient and peaked within 2 – 4 hours post-dose followed by a decrease. IL-11 remained increased at 8 hrs post-dose (186).

When B6C3F1 mice (8 – 10 weeks old) and weanling B6C3F1 mice (3 – 4 weeks old, 5 – 8 each sex/group) were treated per os with 0 or 5 mg DON/kg bw, the maximum plasma DON concentration in weanling mice was double of that in adult mice. In addition, the levels of splenic TNF-α, IL-1β and IL-6 mRNA expression in weanling mice were two- to threefold higher than those in adult mice (53).

Effects of DON on the mRNA expression of suppressor of cytokine signaling (SOCS), proteins considered to suppress cytokine signaling and growth hormone signaling, were studied in B6C3F1 mice (4 – 5 females/group). The animals were given a single dose of 0, 0.1, 0.5, 1, 5 or 12.5 mg DON/kg bw and were analyzed for the mRNA expression of SOCS 1, SOCS2 and SOCS3. A dose-dependent increase in SOCS3 mRNA expression in muscle tissue, spleen and liver were observed in DON treated mice at doses above 0.5 mg/kg bw. At 12.5 mg DON/kg bw, the plasma DON concentration peaked 1 hr after the exposure, while the TNF-α and IL-6 plasma levels peaked after 2 hrs. In the spleen and liver, the TNF-α and IL-6 mRNA expression reached peak levels at 1 – 2 hrs post-dose, while the SOCS3 mRNA expression reached peak level at 2 hrs. Hepatic SOCS3 protein expression was observed from 3 hrs after exposuer by immunohistological staining. Analysis of the mRNA expression of IGFALS (insulin-like growth factor binding protein, acid labile subunit), a downstream molecule in the growth hormone signaling, showed the 75% surpression of hepatic IGFALS expression in DON-treated mice at 3 – 5 hrs post-exposure (187).

When B6C3F1 mice (6 – 8 females/group, 3 – 6 weeks old) were fed diets containing DON at a concentration of 20 mg/kg for 8 weeks, suppressed weight gain was observed compared with the non-treated mice. In the DON-fed mice, the plasma DON concentration increased to 48 ng/mL within 2 weeks and maintained at roughly the same levels (44 – 63 ng/mL) up to week 8. Exposure to DON reduced the hepatic IGFALS mRNA expression to 37% of that of the mice fed control diet by week 2, and retained it at low levels up to week 8. The plasma IGF1 (insulin-like growth factor 1) and IGFALS levels of the DON-fed mice were reduced to 74 – 64% and 34 – 40% than those of the control mice during weeks 2 to 8, respectivery. When B6C3F1 mice (5 females/group) were given a single dose of 0, 0.1, 0.5, 1, 5 or 12.5 mg DON/kg bw, a dose-dependent increase of the hepatic IGFALS mRNA expression at doses above 0.5 mg/kg bw was seen 2 hrs after exposure (188).

d. Apoptosis in lymphoid tissues

In a study on effects of DON on apoptosis in lymphoid tissues, DON (0.1 – 50 μg/mL) exhibited *in vitro* inhibition of dexamethasone-induced apoptosis in mouse thymus-, spleen- and Peyer's patch-derived T cells. Apoptosis of spleen- and Peyer's patch-derived B cells was inhibited by low concentrations of DON but was slightly promoted by high concentrations of DON (189).

When J774A.1 cells were cultured *in vitro* in the presence of DON (10 – 100 μM), apoptosis was induced in a dose-dependent manner (83).

(ii) Hematotoxicity

When ICR mice (10 each sex/group) were fed diets containing purified DON at a dose of 0.5 mg/kg bw for 14 days, RBC count tended to decrease in the DON-fed mice (116).

In the case of Wistar rats (5 males/group) treated for 8 days with gavage containing 0, 0.83, 2.5 or 7.5 mg DON/kg bw per day, plasma haptoglobin levels decreased significantly in rats received DON at dose of or above 2.5 mg/kg bw/day. While, IgG and IgA levels decreased after DON treatment at and above 0.83 mg/kg bw per day and at 7.5 mg/kg bw per day, respectively (169).

The hemolytic effect of DON on rat RBCs was investigated *in vitro*, at concentrations of 130, 200 and 250 μg/mL. While complete hemolysis was observed at 200 and 250 μg/mL, the hemolytic activity was inhibited by mannitol, glutathion, ascorbic acid, α-tocopherol and histidine. These results suggested that the hemolytic effects of DON might involve any of the following three mechanisms: penetration of phospholipid bilayer and activities at the intracellular level; interactions with cellular membranes; and free radical-mediated phospholipid peroxidation (190).

(iii) Other toxicities

When human lymphocytes were cultured in the presence of 0, 30, 60 or 400 ng/mL of DON for up to 72 hrs, cell proliferation was inhibited by 8, 19 and 99% at 30, 60 and 400 ng/mL respectively. Determination of the expression of CD69, CD25 and CD71, which are cell surface antigens related to lymphocyte activation, revealed that the expression of CD69 diminished at 6 hrs followed by an increase, suggesting inhibition of CD69 expression. The expression of CD25 was observed after treatment with DON at concentrations below

the IC_{50} value, but was conversely suppressed at 400 ng/mL. The effect on the expression of CD71 was similar to that on CD25 in many respects. It was therefore considered that DON suppresses cell proliferation mainly before, or at an early stage of, CD25 expression by lymphocytes (191).

In an examination of effects of DON on the colony forming activity of CFU-GM (colony forming units for granulocytes and macrophages), hematopoietic progenitor cells isolated from rat bone marrow cells were exposed to 0, 3, 30 or 300 ng/mL of DON. No toxicity was observed at 3 ng/mL (192).

In another study, human umbilical cord blood-derived and rat bone marrow-derived granulocyte-macrophage progenitor cells (GM) were cultured in the presence of DON (10^{-6} – 10^{-8} M) and colony forming activity was studied for 14 days. The results showed that DON in the dose range from 1 x 10^{-6} to 2.5 x 10^{-7} M inhibited human and rat CFU-GM in a dose-dependent manner. The IC_{50} values at days 7, 10 and 14 were 3 x 10^{-8}, 2.9 x 10^{-8} and 3.9 x 10^{-8} M, respectively, in human GM and 2.6 x 10^{-7}, 1.5 x 10^{-7} and 1.6 x 10^{-7} M, respectively, in rat GM. The toxicity of DON in human GM and rat GM was approximately 1/10 and 1/100, respectively, of that of T-2 toxin or HT-2 toxin (193).

When human hematopoietic progenitor cells were exposed to 0, 3, 90 or 300 ng/mL of DON, the colony forming activity was inhibited by DON at 90 ng/mL or above. At 3 ng/mL, inhibition of colony formation was found on day 7, but this effect disappeared after 10 and 14 days of culture. These results suggested that hematological lesions found in human may be caused by destruction of hematopoietic progenitor cells (194).

On the colony forming activity of erythroid progenitors isolated from human peripheral blood, 3 – 75 ng/mL of DON exhibited effects that were similar in levels to those on human CFU-GM, suggesting that erythroid progenitors are target cells of DON (195).

An analysis of the effects of low concentrations (0 – 200 ng/mL) of DON on the structural and functional charateristics of Caco-2 cells and T84 cells (a human gastrointestinal tract-derived cell line) showed morphological abnormalities in Caco-2 cells, in which brush borders were reduced and microvilli were extended or shortened. DON also reduced the transepithelial electrical resistance (TEER) of Caco-2 and T84 cells and increased the permeability of a pigment (lucifer yellow) through gap junctions. Caco-2 cells showed decreased alkaline phosphatase and sucrase-isomaltase activities. These results suggest that DON may have structural and functional effects on intestinal cell differentiation (196).

In Caco-2 cells and IPEC-1 cells (a porcine gastrointestinal tract-derived cell line), DON reduced TEER and increased the permeability to 4 kDa dextran and to pathogenic *Escherichia coli*. These changes in barrier functions were associated with a specfic decrease of claudin proteins, which are intracellular adhesion molecules. A decrease in claudin-4 proteins was also observed *in vivo* in the jejunum of piglets exposed to 2.85 mg DON/kg feed for 5 weeks (197).

After *ex vivo* exposure of the intestines of 4- to 5-week-old piglets to 1 μM DON for 4 hrs, shortened or adhered villi, small intestinal cell lysis, edema, etc. were studied , but no effects were observed. (198).

The effects of DON or NIV (each, 0 – 1,000 ng/mL) on NO production induced by LPS (lipopolysaccharide) stimulus were investigated *in vivo* using RAW264 cells (a mouse monocytic leukemia-derived cell line). DON and NIV suppressed, in a dose-dependent manner, the production of inducible NO synthase (iNOS) and the function of IFN-β, resulting in the decreased NO production (199).

To investigate the effects of fish oil with high DHA (docosahexaenoic acid) content on DON-induced IL-6 expression, peritoneal macrophages were cultured in the presence of 250 ng/mL of DON. DON-induced IL-6 expression that peaked at 3 hrs was suppressed by knockdown of cAMP response element-binding protein (CREB), a transcription factor, or by suppression of the CREB kinases Akt1/2, MSK1 and RSK1. Suppression of the double-stranded RNA-activated protein kinase (PKR) impaired not only IL-6 expression but also the phosphorylation of CREB and its upstream kinases Akt1, MSK1 and RSK1. On the other hand, peritoneal macrophages harvested from mice treated for 6 – 8 weeks with high DHA content fish oil showed a marked decrease in the phosphorylation of PKR, CREB kinases and CREB. Mice treated with a DHA-rich diet showed suppression of protein phosphatases 1 and 2A. These findings suggest that DON induces PKR- and CREB-dependent IL-6 expression, and that the kinase activities required for these pathways were suppressed in macrophages from mice recieved long-term treatment with DHA (200).

RAW264.7 cells were treated with DON (0 – 1,000 ng/mL) in order to test the hypothesis that PKR is a transmitter upstream of the DON-induced ribosomal toxic stress response. DON induced dose-dependent phospholyration of JNK1/2, ERK1/2 and p38 within 5 min after its addition to the medium, and activated PKR within 1 – 5 min. DON-induced apoptosis was almost totally inhibited in PKR knockdown cells

(201).

B. Nivalenol (NIV)

(1) Acute toxicity

Table 11 shows the oral 50% lethal dose (LD_{50}) of NIV.

Table 11 LD_{50} in acute per os administration of nivalenol (NIV)

Species, strains, sex, age	LD_{50} (mg/kg bw)	Reference
Mouse, ddY, M, 6-week old	38.9	202
Rat, F344, M and F, 5-week-old	19.5	203

The oral, intraperitoneal, subcutaneous and intravenous LD_{50} of NIV in 6-week old male ddY mice were 38.9, 7.4, 7.2 and 7.3 mg/kg bw, respectively. Following oral treatment, marked congestion and hemorrhage were observed in the intestine. Most deaths occurred within 3 days (202).

The oral and subcutaneous LD_{50} values of NIV in F344 rats were 19.5 and 0.9 mg/kg bw, respectively, with diarrhea and congestion in the lungs and gastrointestinal tract being observed (203).

Emesis was observed in ducks treated subcutaneously with 1.0 mg NIV/kg bw or 0.4 mg 4-AcNIV/kg bw (204).

When cats were treated subcutaneously with 1.0 mg 4-AcNIV/kg bw, emesis was observed at 30 min and death occurred at 1 day post-treatment (205).

Intravenous injection of 4-AcNIV at the dose of 0.1 mg/kg bw in dogsevoked emesis in 1 out of the 4 dogs (204).

(2) Subacute toxicity

Table 12 shows the summary of results from subacute toxicity studies using purified NIV.

(i) Mouse

A subacute toxicity study was conducted in which C57BL/6 mice (6 females/group) were fed diets containing NIV at a concentration of 0, 5, 10 or 30 mg/kg for 24 days. A significant decrease in red blood cell count and a slightly decreased number of white cell count were observed in mice in the 30 mg NIV/kg feed dose group. No significant changes were observed on any of the other hematological parameters, feed consumption, weight gain or organ weights in mice in those group. In the 30 mg/kg feed dose group, polyribosomal breakdowns in bone marrow cells were observed by electron microscopy. The NOAEL was 10 mg/kg feed (corresponding to 1.2 mg/kg bw per day, as estimated by SCFJ) (206).

When C54B16 mice (10 males/group) were orally given NIV at a dose of 0, 0.014, 0.071, 0.355, 1.774 or 8.870 mg/kg bw 3 days a week for 4 weeks, the highest dose resulted in a significant decrease in plasma urea, significant increases in plasma alkaline phosphatase activity and plasma IgG levels and a trend to increase in plasma phosphate. The NOAEL was 0.76 mg/kg bw per day (as converted to a per day value) (96).

C57BL/6 mice (10 each sex/group) were fed diets containing NIV at a concentration of 0, 6, 12 or 30 mg/kg feed for 4 or 12 weeks. The NIV used in this study was prepaired by mouldy polished rice on which *F. nivale* had been cultured. According to literature, *F. nivale* produces no trichothecenes other than NIV when cultured on rice. A dose-dependent suppression of body weight gain was shown as follows: significantly decreased body weight in males fed 6 or 30 mg/kg feed for 4 weeks and fed more than 12 mg/kg feed for 12 weeks; in females fed more than 12 mg/kg feed for 4 and 12 weeks. There was a dose-dependent increase in serum alkaline phosphatase activity. While no macroscopic or histological abnormalities were observed, adipose tissue was decreased. The LOAEL was 6 mg/kg feed (corresponding to 0.7 mg/kg bw per day, as estimated by SCFJ) (207).

Table 12 Subacute toxicity of nivalenol (NIV) in-feed

Description of Target (Number per group).	Route (solvent) Duration of dosing	Dose (mg/kg diet)	Dose (mg/kg bw/day)	Effects	LOAEL (mg/kg bw/day)	NOAEL (mg/kg bw/day)	Comments	Reference
Mouse, C57BL/6, 6-week-old (6 females/ group)	Diet, 24 days	0, 5, 10, 30	0, 0.6, 1.2, 3.5*	- At 30 mg/kg feed, decreased number of red cell count, a trend for decreased number of white cell count; damage to polyribosomes in bone marrow cells.	3.5*	1.2*	*F.nivale* moulded rice.	206
Mouse, C54B16, 7-week-old (10 males/ group)	Gavage (solvent: 5% gum arabic water solution) 3 days a week for 28 days		3 days/ week at 0, 0.014, 0.071, 0.355, 1.774, 8.870 mg/kg bw	- At 8.870 mg/kg bw/day, increased plasma phosphate; decreased plasma urea; increased plasma alkaline phosphatase activity; increased IgG.	3.8***	0.76***		96
Mouse, C57BL/6, 7-week-old, (10 each sex/group)	Diet, 4 or 12 weeks	0, 6, 12, 30	0, 0.7, 1.4, 3.5*	- Reduced feed uptake; Reduced body- weight gain;dose-dependent increase in serum alkaline phosphatase activity; reduced adipose tissue.	0.7*		*F.nivale* moulded rice.	207
Rat, Sprague-Dawley, 6-week -old (5 males/ group)	Diet, 14 or 28 days	0, 6, 12	0, 0.6, 1.2**	- At and above 6 mg/kg feed, decreased feed uptake (during initial treatment); changes in organ weights; increased liver microsomal CYP2B1/2; a slight induction of CYP1A2.	0.6**			208
Rat, F344, 5-week-old (12 each sex/group)	Gavage (solvent: distilled water), 30 days		0, 0.4, 2.0	- No abnormalities were found in hematology or serum biochemistry tests. - At 2.0 mg/kg bw/day, a significant increase in liver and spleen weights; no changes were observed in histopathological examination.	2.0	0.4		203
Rat, F344, 6-week-old (10 each sex/group)	Diet, 90 days	0, 6.25, 25, 100	0, 0.4, 1.5, 6.9	- At and above 1.5 mg/kg bw, decreased body-weight.	1.5	0.4		209
Rat, F344, 6-week-old (10 each sex/group)	Diet, 90 days	0, 6.25, 25, 100	0, 0.4, 1.5, 6.9	- At and above 100 mg/kg feed, reduced body- weight; loose stool; thymal atrophy;, decreased number of hematopoietic cells in the bone marrow; diffuse hypertrophy of basophilic cells with increase of castration cells in the anterior pituitary; increase of ovarian atretic follicles. - In males at and above 25 mg/kg feed, decreased body-weight.	0.4			210

Description of Target (Number per group).	Route (solvent) Duration of dosing	Dose (mg/kg diet)	Dose (mg/kg bw/day)	Effects	LOAEL (mg/kg bw/day)	NOAEL (mg/kg bw/day)	Comments	Reference
				- In females at and above 6.25 mg/kg feed, decreased number of white blood cells.				
Pig, 51-day old (6 males/ group)	Diet, 21 days	0, 2.5, 5		- In some animals, gastrointestinal erosion and nephropathy. - At 5 mg/kg feed, decreased number of splenic cells. - At 2.5 mg/kg feed, a trend to time-dependent increase in IgA production.				211
Chicken, 7-day old (6 males/ group)	Diet, 20 days	Study I: 0, 0.5, 2.5, 5. Study II: 0, 3, 6, 12.		Study I: - At 2.5 and 5 mg/kg feed, increased plasma urea acid levels. Study II: - At 6 and 12 mg/kg feed, reduced weight gain; reduced feed consumption uptake; reduced feed conversion. - At and above 3 mg/kg feed, gizzard erosion.				212
Layer chicken (White Leghorn), 55-week old (5 females/ group	Diet, 50 days	0, 1, 3, 5		- At 5 mg/kg feed, decreased plasma alkaline phosphatase; decreaced total protein and glucose. - At 3 and 5 mg/kg feed, gizzard erosion; hemorrhages in the duodenum; swollen cloaca; oviducts with immature eggs. - At 1 mg/kg feed, light, enlarged and fragile livers.				90

*: Converted values based on the SCF standards.
**: The intake was estimated using the relevant conversion factors.
***: The 3-times-weekly doses were converted to daily doses.

(ii) Rat

Sprague-Dawley rats (5 males/group) were fed diets containing NIV at a concentration of 0, 6 or 12 mg/kg feed for 2 or 4 weeks. Rats received diets containing the 6 mg NIV/kg feed and higher exhibited an clear decrease in feed consumption at 1 and 2 weeks post-treatment, followed by recovery at 4 weeks. In rats given a dose of 12 mg/kg feed for 2 weeks significant decreases in the absolute and relative weights of the liver and spleen were observed. Rats given at a dose or above 6 mg NIV/kg feed for 4 weeks exhibited a significant increase in the relative weights of the liver and kidneys, while rats at a dose of 12 mg NIV/kg feed exhibited a significant decrease in the absolute and relative weights of the spleen. A transient increase in CYP2B1/2 as well as a slight induction of CYP1A2 was seen in hepatic microsomes of NIV fed mice. The LOAEL based on decreased organ weights was 6 mg/kg feed (i.e., 0.6 mg/kg bw per day[8]) (208).

[8] The intake was estimated using the relevant conversion factors used by JECFA (IPCS: EHC70).

Species	Final body weight (kg)	Intake (g/animal/day)	Intake (g/kg bw/day)
Rat	0.1	10	100

A sub-acute toxicity was studied in F344 rats (12 each sex/group) treating by gavage at the dose of 0, 0.4 or 2.0 mg NIV/kg bw per day for 30 days. Rats terated with the dose of 2.0 mg/kg bw per day exhibited a trend for decreased and increased body weight in males and females, respectively, both without significant differences. No abnormalities were found in hematology or serum biochemistry tests. In rats given 2.0 mg/kg bw per day, liver and spleen weights were significantly increased, while no changes were observed on histopathological examination (203).

F344 rats (10 each sex/group) were fed diets containing NIV at doses of 0, 0.4, 1.5 or 6.9 mg/kg bw per day for 90 days. Rats given more than 1.5 mg/kg bw/day exhibited reduced body weight. While NK activity was increased in rats received the dose of 0.4 mg/kg bw per day and higher. The LOAEL, based on decreased body weight, was 1.5 mg/kg bw per day (209).

To study a sub-acute toxicity, F344 rats (10 each sex/group) were fed diets containing NIV at a concentration of 0, 6.25, 25 or 100 mg/kg feed for 90 days. Males fed diets with the 25 mg NIV/kg feed and above and females fed diets containing NIV of 100 mg/kg feed showed a significant decrease in body weight. Both males and females fed diets with 100 mg NIV/kg feed showed a significant decrease in the absolute weights of the spleen, kidneys, etc. Males in the same group also exhibited a significant decrease in the absolute and relative weights of the thymus. A significant decrease in white blood cell count was observed in males fed diets with 100 mg NIV/kg feed and in females fed diets containing NIV at 6.25 mg/kg feed and higher. In both males and females received diets containing 100 mg NIV/kg feed, platelet and red blood cell counts were decreased significantly. Hemoglobin concentration was also significantly decreased in females fed diets containing the same concentration of NIV, 100 mg/kg feed. Histological effects found in both males and females in the 100 mg/kg feed dose group were thymal atrophy, hypocellularity in the bone marrow, diffuse hypertrophy of basophilic cells with increases of castration cells in the anterior pituitary, and increases of ovarian atretic follicles. The LOAEL was 6.25 mg/kg feed (corresponding to 0.4 mg/kg bw per day) (210).

(iii) Pig

In pigs (6 males/group) fed diets containing purified NIV at concentrations of 0, 2.5 or 5 mg/kg for 21 days, no feed refusal, emesis nor signs of changes in general condition as well as no changes in body or organ weights were observed. On autopsy, some of the NIV-treated animals had gastrointestinal erosion and nephropathy. There was a dose-dependent decrease in splenic cell number. Animals treated with NIV at the dose of 2.5 mg/kg feed exhibited a trend for time-dependent increase in IgA production and a tendency for decreased IgG production (211).

(iv) Chicken

When chickens (6 males/group) were given diets containing NIV at a concentration of 0, 0.5, 2.5 or 5 mg/kg for 20 days, increased levels of plasma urea were observed in chickens received the dose of 2.5 and 5 mg/kg feed. In another study, conducted under the same conditions except that NIV was fed at a concentration of 0, 3, 6 or 12 mg/kg feed, decreased body weight gain and an approximately 6% decrease in feed consumption and efficiency were seen in chickens treated with 6 and 12 mg NIV/kg feed. Gizzard erosion was evoked in chickens treated with 3 mg NIV/kg feed and higher dose (212).

In layer chickens (White Leghorn, 5 females/group) fed diets containing NIV at a concentration of 0, 1, 3 or 5 mg/kg for 50 days, there was a decrease in feed consumption without any changes in body weight or egg productivity or quality. Decreased plasma alkaline phosphatase, total protein and glucose were observed in chickens fed 5 mg NIV/kg feed. Forty to 75% of the hens in the groups received NIV at 3 and 5 mg/kg feed dose showed gizzard erosion, hemorrhages in the duodenum, swollen cloaca, and oviducts with immature eggs. Some light, enlarged and fragile livers were observed in chickens in the 1 mg/kg feed dose group (90).

(3) Chronic toxicity and carcinogenicity

(i) Chronic toxicity studies

Table 13 shows the sumary of studies on chronic toxicity of NIV.

A chronic toxicity of NIV was studied in 7-week-old C57BL/6 mice (6 females/group) where the animals were fed diets containing NIV at a concentration of 0, 6, 12 or 30 mg/kg (corresponding to 0, 0.68, 1.51 or 3.84 mg/kg bw per day, respectively) for 1 year. The NIV used in this study was prepaired by moldy

polished rice on which *F. nivale* had been cultured. According to literature, *F. nivale* produces no trichothecenes other than NIV when cultured on rice, in which AcNIV was not detected. Treatment of Rats with NIV-containing diets, at all the doses,resulted a dose-dependent decrease in body weight and feed consumption. Rats in the NIV-fed groups showed a decrease in the net weights of the liver, kidneys and thymus as well as a significant dose-dependent increase in the relative weights of the liver, kidneys, thymus and spleen. Macroscopic and histological observations indicated no abnormalities in the liver, thymus, spleen, kidneys, stomach, adrenal glands, pituitary gland, ovaries, sternum, bone marrow, lymph node, brain or small intestine. A significant decrease in the number of white blood cell was seen in rats recieved NIV at dose of 30 mg/kg feed at 6 months and in rats received 6 mg NIV/kg feed or higher at 1 year. The LOAEL was 6 mg/kg feed (corresponding to 0.68 mg/kg bw per day) (202).

A chronic toxicity was examined also in 7-week-old C57BL/6 mice (42 females/group), feeding animals with diets contanining NIV at a concentration of 0, 6, 12 or 30 mg/kg (corresponding to 0, 0.66, 1.38 or 3.49 mg/kg bw per day, respectively) for 2 years. The NIV used in this study was prepared by moldy polished rice on which *F. nivale* had been cultured. According to literature, *F. nivale* produces no trichothecenes other than NIV when cultured on rice, in which AcNIV was not detected. Decreased body weight and a dose-dependent decrease in feed consumption were shown at all doses examined. Net weight of liver was decreased in mice treated with NIV at 30 mg/kg feed. A significant decrease in absolute kidney weight was observed at and above 12 mg NIV/kg feed. There were dose-dependent and significant increases in both serum alkaline phosphatase and non-esterified fatty acid levels in mice treated with NIV of 30 mg/kg feed. Macroscopic and histological examinations found no tumor attributable to NIV in any of the dose groups. Spontaneous occurring tumors, mostly lymphomas, were of common incidence in all groups. In the mice treated with NIV of 30 mg/kg feed, lymphoma developed later and grew slowly. There were sporadic occurrence , at a low rate, of amyloidosis in the small intestinein mice of the 12 and 30 mg/kg feed dose groups. The LOAEL was 6 mg/kg feed (corresponding to 0.66 mg/kg bw per day) (213).

Table 13 Chronic toxicity of nivalenol (NIV)

Description of Target (Number per group)	Route (Solvent), duration period	Dose		Effecs	LOAEL (mg/kg bw/day)	NOAEL (mg/kg bw/day)	Comments	Reference
		(mg/kg diet)	(mg/kg bw/day)					
Mouse, C57BL/ 6CrS1c, 7-week-old, (6 females/ group)	Diet, 1 year	0, 6, 12, 30	0, 0.68, 1.51, 3.84	- At 30 mg/kg diet after 6 months and all NIV doses after 1 year: a significant decrease in number of white blood cells; a dose-dependent decrease in the net weights of, and an increase in the relative weights of, the liver, kidneys and thymus. - No histological abnormalities were found.	0.7		*F.nivale* moulded rice.	202
Mouse, C57BL/ 6CrS1c, 7-week -old, (42 females/ group)	Diet, 2 years	0, 6, 12, 30	0, 0.66, 1.38, 3.49	- At all doses, decreased body weight. - At 12 and 30 mg/kg feed, decreased net weight of kidney. - Only at 12 mg/kg feed, decreased kidney weight; a dose-dependent increase in serum alkaline phosphatase and non-esterified fatty acid levels. - No NIV-induced tumors were found.	0.7		*F.nivale* moulded rice.	213

(ii) Other studies

In order to investigate the effect of NIV on the induction of hepatocellular carcinoma by aflatoxin B_1 (AFB1), 1-week-old C57B1/6×C3HF$_1$ mice (15 – 26 each sex/group) were treated with AFB1 intraperitoneally at the dose of 6 mg/kg bw, followed after 6 weeks by 1-year feeding with diets containing NIV at a concentration of 0, 6 or 12 mg/kg. The NIV used in this study was prepared by moldy polished rice on which *F. nivale* had been cultured. According to literature, *F. nivale* produces no trichothecenes other than NIV when cultured on rice, in which AcNIV was not detected. While hepatocellular carcinoma and adenoma occurred in male mice of all three groups, the incidences in females were 31%, 21% and 0% in mice treated with 0, 6 and 12 mg NIV/kg feed, respectively (214).

A medium-term study on hepatocarcinogenicity was conducted in F344 rats (4 – 16 males/group). In this study, rats were given a single intraperitoneal dose of diethylnitrosoamine (DEN), followed by another single intraperitoneal dose of AFB1 after 2 weeks. Then, rats were fed with diets containing NIV at a concentration of 6 mg/kg feed (corresponding to 0.6 mg/kg bw per day[9]) for 6 weeks. The NIV used in this study was prepared by moldy polished rice on which *F. nivale* had been cultured. According to literature, *F. nivale* produces no trichothecenes other than NIV when cultured on rice, in which AcNIV was not detected. The animals received partial excision of the liver at week 3 after commencement of the study and liver sections at week 8, then GST-P (glutathione S-transferase placental type) expression in hepatic foci, an indicator of pre-cancerous lesion, was examined. There were no marked changes in the group treated with NIV alone or the group treated with both NIV and DEN. DEN and AFB1 caused a marked increase in GST-P-positive cells in rats, while the rats treated with combination of DEN, AFB1 and NIV showed an increase in the numbers and areas of GST-P-positive foci (215).

(4) Reproductive/developmental toxicity

Table 14 shows the summary of reproductive/developmental toxicity of NIV.

To examine reproductive/developmental toxicity of NIV, ddN mice (at least 3 males/group) were treated subcutaneously, intraperitoneally or orally with 0 or 0.4 – 60 mg NIV/kg bw per day. Treatment with NIV resulted in a decrease in the number of spermatogenic cells and partial necrosis of sperm cells. Multinuclear giant cells were found in the testes (doses unspecified) (216).

Pregnant ICR mice (10 – 11 females/group) were fed diets containing NIV-contaminated moldy rice at the NIV-concentration of 0, 6, 12 or 30 mg/kg during days 0 – 18 of gestation. In the 30 mg/kg feed group, mother animals exhibited a significant suppression of weight gain, while their fetuses showed a significant decrease (82.6%) in survival rate as well as delayed ossification of the vertebrae. Treatment with NIV of 12 mg/kg feed and higher dose resulted a significant decrease in fetal body weight. In other experiment, pregnant ICR mice (5 – 10 females/group) were given gavage containing purified NIV at the doses of 0, 1, 5, 10 or 20 mg/kg bw/day during days 7 – 15 of gestation. In the 10 mg/kg bw per day and higher dose groups, mother animals showed a significant suppression of weight gain and increased stillbirths and late fetal resorption. In the 5 mg/kg bw per day and higher dose groups, retardation of fetal intrauterine weight gain was observed. No teratogenicity was found (217).

Table 14 Reproductive/developmental toxicity of nivalenol (NIV)

Description of Target (Number per group)	Route (solvent), duration period	Dose (mg/kg diet)	Dose (mg/kg bw/day)	Findings/Effects	LOAEL (mg/kg bw/day)	NOAEL (mg/kg bw/day)	Comments	Reference
Mouse, ICR (10 – 11 females/group)	Diet, days 0-18 of gestation	0, 6, 12, 30	0, 0.7, 1.4, 3.5*	- At 30 mg/kg feed, maternal weight gain suppression; embryotoxicity.	1.4*	0.7*	*F.nivale* moulded moldy rice	217

[9] The intake was estimated using the relevant conversion factors used by JECFA (IPCS: EHC70).

Species	Final body weight (kg)	Intake (g/animal/day)	Intake (g/kg bw/day)
Rat	0.1	10	100

				- At and above 12 mg/kg feed, fetal growth suppression.				
Mouse, ICR (5 – 10 females/group)	Intragastric (saline), days 7-15 of gestation		0, 1, 5, 10, 20	- At and above 10 mg/kg bw/day, maternal weight gain suppression; embryotoxicity. - At and above 5 mg/kg bw/day, fetal growth suppression.	5	1		217

*: Converted values by SCFJ.

(5) Genotoxicity

Table 15 shows a summary of genotoxicity of NIV.

In an *in* vitro study using V79-E cells (a Chinese hamster lung-derived cell line), NIV induced cell cycle retardation. In the presence of the metabolic activation system (+S9 mix), slight chromosomal aberrations were seen. A slight increase in sister chromatid exchange (SCE) frequency was observed. These observed effects were nonspecific, suggesting that they were caused by inhibition of protein synthesis (218).

In a chromosome aberration test using V79 cells, NIV purified from contaminated corn induced a 2- to 3-fold increase in chromosomal aberrations at 0.001 – 0.03 µg/mL, as compared with the solvent-treated control (143).

In another chromosome aberration test using V79 cells, NIV purified from contaminated wheat, barley or corn induced a 2- to 3-fold increase in chromosomal aberrations at 0.03 µg/mL of each, as compared with the control: each at a frequency of less than 5% (144).

In a short-term transformation assay using v-Ha-ras transfected BALB/3T3 cells, NIV showed no initiation or promotion activity (147).

In a single-cell gel electrophoresis assay (comet assay) of NIV using CHO cells and ICR mice (4 males/group), NIV at 50 and 100 µg/mL induced DNA damage of CHO cells in the absence of a metabolic activation system. In an *in vivo* comet assay, 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 (219).

Transgenic (Tg) mice (Muta™ Mouse) were treated by gavage with NIV, and were examined for mutations in organs. All organs examined were negative. On the other hand, a comet assay showed positive results with in some organs[10] (220).

Table 15 Results of assays for the genotoxicity of nivalenol (NIV)

A: *in vitro*

End-point	Test object	Concentration	Results		Reference
			-S9	+S9	
Sister chromatid exchange	Chinese hamster V79-E cells	5 - 50 µM/plate	Positive (mild)	Positive (mild)	218
Chromosomal aberrations	Chinese hamster V79-E cells	5 - 50 µM/plate	Negative	Positive (mild)*	218
Chromosomal aberrations	Chinese hamster V79 cells	0.001 - 0.03 µg/mL	Positive (3-fold)	—	143
Chromosomal aberrations	Chinese hamster V79 cells	0.03 µg/mL	Positive (3-fold)	—	144
Transformation	v-Ha-ras transfected BALB/3T3 mouse embryo cells	0.01 - 0.2 µg/mL	Negative	—	147
DNA damage (comet assay)	CHO cells	50, 100 µg/mL	Positive	—	219

[10] According to the examiner of relevant study, after mice were treated with NIV in gavage at 0 or 6 mg/kg bw 4 times at weekly intervals, the forestomach, kidneys, urinary bladder, large intestine, lungs, liver, bone marrow and spleen exhibited negative for the induction of mutations. The comet assay showed positive results for the liver and stomach only.

*: All aberrations were daughter chromatid exchange.
-: Not tested.

B: *in vivo*

Endpoint	Test system	Results	Ref.
DNA damage (comet assay)	ICR mice(Male) treated with NIV (20 mg/kg bw)	p.o.: Positive (kidneys, bone marrow, stomach, jejunum and colon) i.p.: Positive(colon only)	219
Gene mutation	Tg- mouse (Muta™ Mouse)	Negative[11]	220
DNA damage (comet assay)	Mouse	Possitive[11]	220

(6) Other toxicities of NIV (immunotoxicity, hematotoxicity, etc.)

(i) Immunotoxicity

a. Effects of NIV on immune responses

When BALB/c mice (10 females/group) were received free access of water containing NIV at a concentration of 0, 0.2, 2 or 6 mg/kg for 4 weeks, NIV exerted no effect on their survival rate in the mice infected with *S. enteritidis* on day 14 (154).

To study effects of NIV on immune reponses in rats, F344 rats (6 each sex/group) were fed diets containing NIV at a concentration of 0, 6.25, 25 or 100 mg/kg (corresponding to 0, 0.4, 1.5 or 6.9 mg/kg bw per day, respectively) for 90 days. Rats received NIV of 25 mg/kg feed or higher showed a significant dose-dependent decrease in the splenic T lymphocyte/B lymphocyte ($CD3^+/B220^+$) ratio, while those recieved 100 mg NIV/kg feed showed a significant increase in the $CD4^+$ T lymphocyte (helper T lymphocyte)/$CD8^+$ lymphocyte (cytotoxic T lymphocyte) ratio. A significant increase in NK activity was observed at all NIV levels examined (209).

b. Changes in serum IgA levels and IgA nephropathy

As is the case with DON, NIV has been reported to have effects on IgA and, in mice, to be associated with IgA nephropathy (Table 16).

When C57BL/6 mice (10 males/group) were given NIV (solvent: 5% arabic gum solution in water) orally by gavage with 0, 0.014, 0.071, 0.355, 1.774 or 8.870 mg/kg bw 3 days weekly for 4 weeks. A significant increase in plasma IgG but no changes in plasma IgA was observed in mice received 8.870 mg NIV/kg bw (96).

In C57BL/6 mice (10 males/group) that were given NIV (solvent: 5% arabic gum solution in water) by gavage containing 0, 0.071 or 0.355 mg NIV/kg bw 3 days weekly for 4 weeks, a significant increase in plasma IgA was observed with the dose of NIV 0.071 mg/kg bw or above (168).

When C3H/HeN, C3H/HeJ and BALB/c mice (9 – 12 females/group) were fed diets containing purified NIV at a concentration of 0, 6 or 12 mg/kg feed (corresponding to 0, 0.9 or 1.8 mg/kg bw per day[11], respectively) for 4 or 8 weeks, the NIV-treated animals showed increased IgA accumulation in glomeruli and increased serum IgA with all NIV doses, particularly with NIV of 12 mg/kg feed at 8 weeks (221).

In an immunotoxicity study where BALB/c mice (20 females/group) were given a single dose of 0 or 15 mg NIV/kg bw in gavage, followed by observation of lymphoid organ cells up to 24 hrs post-dose. A significant increase in the number of IgA^+ cells in Peyer's patches was seen after 9 hrs post-dose. In cells isolated from Peyer's patches at 3 hrs post-dose, a significant decrease in the numbers of pan-T, pan-B cells and viable cells were observed. Cells isolated from Peyer's patches at 9 hrs post-dose showed a significant increase in all B cell subpopulations, particularly in IgA^+ B cells. The numbers of IgA+ and IgM+ B cells remained higher than those of the control group thereafter (222).

In a study with OVA-TCR Tg (OVA-specific T-cell receptor transgenic) mice, the animals (4 males/group) were given diets containing OVA either alone or in combination with water containing 0 or 6 mg NOV/kg (corresponding to 0.9 mg/kg bw per day[12]). Treatment with OVA alone resulted in increased serum OVA-specific IgE, IgG_1 and IgA levels as well as total IgE, IgG_1 and IgA levels, whereas combined treatment with OVA and NIV resulted in a significant inhibition of total IgE production and OVA-specific IgE, IgG_1 and IgA production (223).

To examine immunotoxicity, F344 rats (10 each sex/group) were treated with NIV in diets at a dose of 0, 0.4, 1.5 or 6.9 mg/kg bw per day for 90 days. Rats recieved 6.9 mg NIV/kg bw per day exhibited a significant increase in IgM but no changes in IgG and IgA levels (209).

In a study where pigs (6 males/group) were fed diets containing purified NIV at a concentration of 0, 2.5 or 5 mg/kg feed for 21 days, significant differences in plasma IgA levels were not observed between the control and NIV-fed animals. However, animals treated with purified NIV of 2.5 mg/kg feed exhibited

[11] The intake was estimated using the relevant conversion factors used by JECFA (IPCS: EHC70).

Species	Final body weight (kg)	Intake (g/animal/day)	Intake (g/kg bw/day)
Mouse	0.02	3	150

trend for time-dependent increases in IgA production and a trend for decreased IgG production (211).

Table 16 Effects of nivalenol (NIV) on IgA production

Description of Target (Number per group)	Route (solvent), duration period	Dose		Effects	LOEL (mg/kg bw/day)	NOEL (mg/kg bw/day)	Nots	Referece
		(mg/kg feed)	(mg/kg bw/day)					
Mouse, C57BL/6, 6-week-old (10 males/ group)	Oral gavage (5% gum arabic water solution) 3 days a week for 4 weeks		0, 0.014, 0.071, 0.355, 1.774, 8.870 mg/kg bw	- At 8.870 mg/kg bw, increased plasma IgG. - No effects on IgA.		3.8**		96
Mouse, C57BL/6, 6-week-old (10 males/ group)	Oral gavage (5% gum arabic water solution) 3 days a week for 4 weeks		0, 0.071, 0.355 mg/kg bw	- Increased plasma IgA.	0.03**			168
Mouse, C3H/HeN, C3H/HeJ, BALB/c, 6-8-week-old (9-12 females/ group)	Diet, 4 or 8 weeks	0, 6, 12	0, 0.9, 1.8*	- Increased serum IgA. - IgA nephropathy-like immunopathological changes in the kidneys (in association with the increased serum IgA).	0.9*		*F.nivale* moulde d rice	221
Mouse, BALB/c, 5-week-old (20 females/ group)	Single gavage (10% DMSO)		0, 15	- Increased IgA^+ cells in Peyer's patches; decreased pan-T and pan-B cells in lymphoid organs.	15			222
OVA-specific T-cell receptor αβ-Tg mouse, BALB/c, 8-13-week-old, males	Drinking water, 2 or 4 weeks	0, 6	0, 0.9*	- Significant inhibition of OVA-speciric-IgE production and OVA-specific IgE, IgG_1 and IgA production; inhibition of IL-4 production; increased IL-2 production in spleen cells.	0.9*			223
Rat, F344, 5-week-old (10 each sex/group)	Diet, 90 days	0, 6.25, 25, 100	0, 0.4, 1.5, 6.9	- At 6.9 mg/kg bw/day, increased IgM. - Noeffects on IgA or IgG.		6.9		209
Pig, 51-day-old (6 males/ group)	Diet, 21 days	0, 2.5, 5		- No significant differences in plasma IgA compared with the control. - (At 2.5 mg/kg feed, a trend for time-dependent increase in IgA production.)				211

*: The intake was estimated using the relevant conversion factors.
**: The 3-times-weekly doses were converted to daily doses.

c. Cytokine expression

Effects of NIV on cytokine expression was examined in OVA-TCR Tg mice (4 males/group) with free access of water containing 0 or 6 mg NIV/kg in combination with feed containing OVA. Administration of NIV resulted in the inhibition of IL-4 production and the increase of IL-2 production in splenic cells (223).

In female C3H/HeN mice fed with diets containing NIV at concentration of 0 or 12 mg/kg (corresponding to approximately 1.8 mg/kg bw per day[12]) for 8 weeks, those animals fed NIV exhibited a significant increase in IgA-producing cells in Peyer's patch lymphocytes. In those cells, increased expressions of IL-4, IL-5, IL-6, IL-10 and TGF-β (Th2 cytokine) mRNA were observed (224).

In mouse bone marrow-derived dendritic cells that were pretreated with LPS, exposure of the cells to either NIV or DON alone or to both, each at concentrations between 1 and 3 μM, caused suppression of LPS-induced IL-12 and IL-10 production in a dose-dependent manner, but TNF-α production was increased (225).

d. Apoptosis in lymphoid tissues

When BALB/c mice (5 females/group) were treated orally with 0 or 15 mg NIV/kg bw per day, NIV induced apoptosis in Peyer's patches at 3 hrs post-dose as well as in the thymus at 6 hrs post-dose at maximum level. In the thymus, Peyer's patches and mesenteric lymph nodes, apoptosis was induced in $CD4^+$ and $CD8^+$ cells (222).

ICR:CD-1 mice (5 males/group) were treated orally with 0, 5, 10 or 15 mg NIV/kg bw per day and examined the progress of apoptosis of lymphocytes in the thymus, spleen and Peyer's patches at 12, 24 and 48 hrs post-treatment. The number of apoptotic lymphocytes increased in a dose-dependent manner at 12 hrs in both of the thymus and the Peyer's patches, while the maximum level of apoptotic lymphocytes in the spleen was observed at 24 hrs post-treatment (226).

Apoptosis was induced in a dose-dependent manner, when J774A.1 cells were cultured *in vitro* in the presence of NIV (10 – 100 μM) (83).

(ii) Hematotoxicity

C57BL/6CrSlc mice (6 males/group) were fed diets containing NIV at a concentration of 0, 6, 12 or 30 mg NIV/kg feed (corresponding to 0, 0.68, 1.51 or 3.84 mg/kg bw per day, respectively) for examining hematotoxicity of NIV. A significant decrease in white blood cell count was seen in mice at 30 mg/kg after 6 months and at 6 and 30 mg NIV/kg after 1 year. The LOAEL was 6 mg/kg feed (corresponding to 0.7 mg/kg bw per day) (202).

To examine effects of short-term feeding with NIV, C57BL/6 mice (6 females/group) were fed diets containing NIV at a concentration of 0, 5, 10 or 30 mg/kg feed (by the addition of rice cultured with mold) for 24 days. A significant decrease in number of red blood cells and a slight decrease in number of white blood cells were observed in mice received NIV of 30 mg/kg feed (corresponding to approximately 3.5 mg NIV/kg bw per day, as estimated by the SCFJ), without marked changes in any of the other hematological parameters or feed consumption, body weight gain, or liver, spleen or thymus weight (206).

F344 rats (12 each sex/group) were received daily administration of NIV by oral gavage at a dose of 0, 0.4 or 2.0 mg NIV/kg bw per day for 30 days. No significant changes were observed in any of the hematological or biochemical parameters (203).

(iii) Other toxicities

Inhibitory efects of NIV on *in vitro* mitogen-induced proliferation of lymphocytes were examined using lymphocytes isolated from human peripheral blood. NIV inhibited 50% of cell growth at an average concentration of 72 ng/mL (227).

NIV inhibited PHA (IC_{50}: 350 nM)- or pokeweed (PW) (IC_{50}: 270 nM)-induced proliferation of human lymphocytes isolated from human peripheral blood. NIV also inhibited PW-induced immunoglobulin production. DON exhibited the same effects at a similar dose range. Combinations of NIV with T-2 toxin, diacetoxyscirpenol or DON resulted in an additive inhibitory effect on immunoglobulin production (228).

In an *in vivo* study using RAW264 cells to assess the effects of DON or NIV on LPS-induced NO production, NIV significantly suppressed iNOS production at and above 125 μM/mL (199).

In a study in mouse bone marrow-derived dendritic cells (DCs), the cells were stimulated with LPS and

[12] The intake was estimated using the relevant conversion factors used by JECFA (IPCS: EHC70).

Species	Final body weight (kg)	Intake (g/animal/day)	Intake (g/kg bw/day)
Mouse	0.02	3	150

then were treated with either NIV or DON alone or with both, at a concentration between 1 and 3 μM of each. Down-regulation of NO production as well as the expression of MHC class II and of the accessory CD11c molecules were observed without any changes in the expression of co-stimulatory molecule CD86. NIV but not DON induced DC necrosis significantly. Both toxins inhibited LPS-induced IL-12 and IL-10 production in a dose-dependent manner, but enhanced TNF-α production (225).

C. Combined toxicity of DON and NIV

(1) *in vivo*

A combined toxicity was studied in C57BL/6 mice (10 males/group) by treating animals with gavage containing 0, 0.071 or 0.355 mg DON/kg bw (solvent: 5% arabic gum solution in water), either alone or in combination with the same dose of NIV, 3 times weekly for 4 weeks. Combind administration of DON and NIV resulted in additive increase of plasma IgA and of GST activity assayed with dichloronitrobenzene (DCNB) as the substrate, as well as synergistic increase in plasma uric acid levels (168).

(2) *in vitro*

Table 17 shows a summary of the results of *in vitro* combined toxicity studies of DON and NIV.

A study was conducted to assess the suppressive effects of single or combined exposure to DON, NIV, diacetoxyscirpenol (DAS) and T-2 toxin on PHA- or PW-stimulated proliferation of human peripheral lymphocytes *in vitro*. All toxins suppressed lymphocyte proliferation by single-treatment: the individual IC_{50} for PHA- and PW-stimulated proliferation are as follows; NIV, 350 and 270 nM; DON, 430 and 380 nM; DAS, 4.1 and 4.0 nM; and T-2 toxin, 1.4 and 1.1 nM, respectively. The combined inhibitory effects of NIV (1×10^{-7}M) and DON (2×10^{-7}M) were additive and not synergistic. The combined inhibitory effects of DON and T-2 toxin or DAS were similar or slightly lower than those of individual treatment with T-2 toxin or DAS, suggesting that DON could exhibit antagonistic effects (228).

Suppressive effects of fumonicin B_1 (FB1), α-zearalenol (α-ZEA), NIV and DON on mitogen-induced cell proliferation were investigated using swine blood cells treated with concanavaline A (Con A). Alpha-ZEA (0.5 – 20 μM), NIV and DON (0.065 – 2 μM) suppressed proliferation in a dose-dependent manner; NIV exhibited the strongest effect, followed by DON and α-ZEA. FB1 (0.5 – 80 μM) had no effect on cell proliferation. While FB1 and α-ZEA showed a synergistic suppressive effect on cell proliferation, DON and NIV had neither synergistic nor additive effects (229).

To examine pro-apoptotic effects as combined toxicity of DON and NIV, J774A.1 cells were cultured in the presence of NIV (10 – 100 μM) and/or DON (10 – 100 μM), and the apoptosis inducing effects at 72 hrs were detected. Each toxin induced apoptosis after single treatment in a dose-dependent manner, with IC_{50} of 11.2 ± 0.8 and 16.8 ± 0.2μM respectively. Combined treatment with NIV and DON also induced apoptosis, but the effects was not synergetic and the IC_{50} was 14.0 ± 1.9 μM (83).

The inhibitory effects on yeast (*Kluyveromyces marxianus*) growth were compared between different mycotoxins by disk-diffusion assay, using paper disks containing following combinations of fungal toxins: T-2 and HT-2 toxins; T-2 toxin and T-2 tetraol; DON and NIV; and DON and T-2. While the combination of T-2 and HT-2 toxins and that of DON (5 – 50 μg/disk) and NIV (5 – 100 μg/disk) showed synergetic effects at and below 25 μg/plate, the combination of DON and T-2 toxin showed an antagonistic effect (230).

Table 17 Combined effects of deoxynivalenol (DON) and nivalenol (NIV)

Objects	Concentration	Effects	Reference
Human peripheral lymphocytes	NIV: 1×10^{-7} M DON: 2×10^{-7} M	- Additional inhibitory effects of DON and NIV on PHA- or PW-stimulated cell proliferation.	228
Swine blood cells	0.065 - 2 μM each	- Neither additive nor synergic effects of DON and NIV on Con A-stimulated cell proliferation.	229
J774A.1 cells	10 - 100 μM each	- Neither additive nor synergic effects of DON and NIV on apoptosis induction.	83
Yeast (*Kluyveromyces marxianus*)	DON: 5 – 10 μg/plate, NIV: 5 – 100 μg/plate	- At and below 25 μg/plate, synergic inhibition of yeast proliferation	230

3. Findings in humans

(1) Clinical findings

Exposure to DON may result in acute symptoms, such as nausea, vomiting, diarrhea, gastrointestinal upset, headache, dizziness and pyrexia, within 30 min (231). It is difficult to distinguish these symptoms from those associated with gastrointestinal disorders that are likely to be caused by microorganisms, such as *Bacillus cereus*, which produces an emetic toxin (3).

(2) Epidemiological studies, etc.

Table 18 shows a summary of reports from epidemiological studies on DON and NIV.

Table 18 Epidemiological studies, etc. concerning deoxynivalenol (DON) and nivalenol (NIV)

Country	Year	Source	Amount of intake and concentration of contaminant toxin in food	Symptoms	Reference
China (Xingtai, Hebei province)	1984	Moldy corn	- Concentration of DON : 0.34-3.75 mg/kg (GC-MS: 2 samples), 5.10-92.8 mg/kg (RIA: 3 samples) (T-2 not tested; NIV not detected)	362 out of 383 persons (94.5%) had symptoms after 3-30 min, including: nausea (89.8%), dizziness (78.2%), vomiting (61.16%), abdominal pain (6.1%) diarrhea (5.2%), pyrexia (5.5%) and palpitation (0.9%).	Based on data organized by Luo in 1994 (232)
China (Puyang, Henan province)	1985	Fusarium head blight-affected wheat, etc.	- Concentration of DON: 2.0-40.0 mg/kg (TLC: 14 samples) (T-2 and NIV not tested)	101 out of 217 persons (46.5%) had symptoms	
China (Yulin city, Guangxi province)	1989	Wheat flour	- Concentration of DON: 1.5-2.2 mg/kg (TLC: 3 samples) (T-2 and NIV not tested by TLC)	40 out of 160 persons had symptoms	
China (Peishan, Heibei province)	1988	Corn flour	- Concentration of DON: 20.0-50.0 mg/kg (TLC: 3 samples), 2.1-57.9 mg/kg (GC: 6 samples) (T-2 and NIV not tested by TLC and not dected by GC)	270 out of 514 persons (52.5%) had symptoms	
China (Taiyuan, Shanxi province)	1988	Corn flour	- Concentration of DON: 3.0 mg/kg (TLC: 1 sample) (T-2 and NIV not detected)	142 out of 209 persons (67.9%) had symptoms	Based on data organized by Luo in 1994 (232)
China (Hengxian, Guangxi province)	1989	Corn flour	- Concentration of DON: 4.0-36.0 mg/kg (TLC: 5 samples) 59.3-66.8 mg/kg (GC: 2 samples) (T-2 and NIV not tested by TLC and not detected by GC)	10 out of 10 persons had symptoms	
China (Anhui province)	1991	Moldy wheat	- Concnentration of DON: 2.0-50 mg/kg (TLC: 10 samples) (T-2 and NIV not tested)	130 and 141 persons had symptoms	
China	1990	Comparison of DON intake from corn between patients with esophageal cancer and control patients	- Mean content of DON: 0.57 mg/kg and 0.099 mg/kg in patients with esophageal cancer and control patients, respectively		233

Country	Year	Source	Amount of intake and concentration of contaminant toxin in food	Symptoms	Reference
China	1995	Comparison of exposure to mycotoxins between areas with high esophageal cancer risk and control areas	- Contents of mycotoxins in corn in areas with high esophageal cancer risk vs. control areas: DON; 0.4 mg/kg vs. 0.05 mg/kg 15Ac-DON; 0.24 mg/kg vs. not detected NIV; 0.086 mg/kg vs. 0.059 mg/kg	The occurrence rate of esophageal cancer was correlated with trichothecene and ZEN contents and not with DON or NIV content.	234
China	1993	Comparison of exposure to mycotoxins between areas with high primary liver cancer risk and control areas	- Mean content of DON: 0.89 mg/kg in high-risk areas and 0.49 mg/kg in control areas.		235
China	1992	Research conducted in relation to the occurrence rate of Kashin-Beck disease (endemic osteoarthritis)	- DON content was significantly higher in all high occurrence areas (range: 0.005-3.9 mg/kg) than in low occurrence areas (range: 0.002-0.7 mg/kg). - 15Ac-DON and 3-AcDON contents were also significantly higher.		236
China	2004	NIV content in contaminated crops in areas with high esophageal and gastric cardia cancer risk was measured and compared with U.S. data	- Mean NIV and DON concentrations in wheat, barley and corn were 830 ± 927 µg/kg and 4281 ± 6114 µg/kg, respectively, and were estimated to be 400- to 800-fold of the corresponding mean concentrations in the U.S.		144
India	1987	Ingestion of bread made from rain-damaged wheat	- DON, AcDON, NIV and T-2 toxin contents: 0.34-8.4 mg/kg in 11 out of 24 samples; 0.6-2.4 mg/kg in 4 out of 24 samples; 0.03-0.1 mg/kg in 2 out of 24 samples; and 0.55-4 mg/kg in 3 out of 24 samples. - The LOAEL is estimated to be 0.44 µg/kg bw (note that other toxins are also contained, as mentioned above).	Lower abdominal pain, abdominal distension, dizziness, headache, throat inflammation, nausea, vomiting, diarrhea and bloody stool	237, 238

4. Assessments of institutions and international organizations

(1) The Joint FAO/WHO Expert Committee on Food Additives (JECFA)

In 2000, JECFA conducted an assessment of DON. In a 2-year feeding study in mice, DON showed no carcinogenicity. Although the mean body weight of animals was lower in the lowest dose group (100 µg/kg bw per day) than in the control group, this difference was considered not to be biologically significant. In addition, no other toxicological changes were found in the lowest dose group. Based on these results in a 2-year study in mice, JECFA set the provisional maximum tolerable daily intake (PM-TDI) of DON at 1 µg/kg bw per day, by applying a safety factor of 100 to the NOAEL of 100 µg/kg bw per day. JECFA concluded that this level of intake has no effects on the immune system, growth or reproduction (3).

Subsequently, JECFA conducted a reassessment of DON and published a summary of the assessment results in March 2010. Considering the fact that 3-AcDON is converted to DON *in vivo* and therefore contribute to the total DON-induced toxicity, JECFA decided to convert the PM-TDI for DON to a group PM-TDI of 1 µg/kg bw, including its acetyiated derivatives 3- and 15-AcDON. In this regard, JECFA considered the toxicity of acetyiated derivatives to be equal to that of DON. JECFA set an acute reference dose (ARfD) at 8 µg/kg bw/day by applying a safety factor of 25 to the $BMDL_{10}$ value for emesis in pigs, using the $BMDL_{10}$ at 0.21 mg/kg bw per day estimated by the benchmark dose method (239).

No assessment of NIV was conducted by JECFA.

(2) The International Agency for Research on Cancer (IARC)

In 1993, IARC conducted an assessment of the carcinogenicity of *F. graminearum*-, *F. culmorum*- *and F. crookwellense*-derived toxins (ZEN, DON, NIV and AcNIV) (4).

IARC reported that there was inadequate evidence of the carcinogenicity of *F. graminearum*-derived toxins in humans, and that no data were available on the carcinogenicity of *F. culmorum*- and *F. crookwellense*-derived toxins in humans. In addition, there is inadequate evidence of the carcinogenicity of DON, NIV and AcNIV in experimental animals.

The IARC concluded that these *F. graminearum*-, *F. culmorum*- *and F. crookwellense*-derived toxins are not classifiable as to their carcinogenicity to humans (IARC carcinogenicity classification Group 3).

(3) The European Commission (EC) Scientific Committee on Food (SCF)

The EC-SCF published its opinion on an assessment of DON in 1999, that of NIV in 2000, and its opinion on a group evaluation of T-2 tixin, HT-2 toxin, NIV and DON in 2002 (31, 32, 33).

Since DON was not considered to be carcinogenic or teratogenic, the temporary tolerable daily intake (tTDI) was set at 1 μg/kg bw per day, by applying an uncertainty factor of 100 to the NOAEL of 0.1 mg/kg bw per day based on a chronic dietary study with mice. The EC-SCF concluded that use of this tTDI value would protect against the other subchronic and reproductive effects as well as the acute emetic effect of DON.

A tTDI was set at 0.7 μg/kg bw per day, by applying an uncertainty factor of 1000 to the LOAEL of 0.7 mg/kg bw per day based on a chronic dietary study with mice. The uncertainty factor of 1000 was applied because of the use of a LOAEL and a limited database.

The EC-SCF suspended the setting of a group TDI for T-2 and HT-2 toxins, NIV and DON because the available data were limited to support a group TDI for all trichothecenes assessed.

In the present assessment, the FSCJ, taking into account the ssessment results described above, considered the establishment of a group TDI for DON and AcDON by reviewing the available scientific data. The FSCJ noted that there are limited data about toxicity of 3- and 15-AcDON compared to that of DON (76, 82, 93, 97, 240), and moreover there are some reports suggesting that toxic effects of 3- and 15-AcDON are different from those of DON following a sigle oral administration (98, 102, 97). In addition, while there is one report indicating that 3-AcDON is rapidly metabolized to DON *in vivo* (241), on the otherhand, the metabolism of 15-AcDON *in vivo* has not been elucidated. Based solely on the single report on the metabolism of 3-AcDON mentioned above, the toxicity of 3-AcDON is assumed to be same as DON after being absorbed through the gastrointestinal tract. However, the FSCJ concluded that at the present, the available data, including relative toxicity data, were not sufficient to establish a group TDI for 3- and 15-AcDON and DON.

5. State of exposure

It is known that DON and NIV contaminate mainly grains, such as wheat, barley and corn. The contamination levels of those toxins have been found to be very low in rice, which is the primary staple food of diet in Japan and is consumed in large amounts (242). In addition, only a limited number of cases, where DON or NIV was detected in grains such as rye, oats or rice, has been reported by EU and Codex (243, 244). Wheat, which is consumed in amounts comparable to rice, is likely to be the main source of exposure to DON and NIV in people in Japan, therefore, surveillance of contamination and studies on exposure assessment have been conducted focusing on wheat.

(1) Survey on conditions of contamination

Following the establishment of a temporary regulation limit of DON in unpolished wheat (1.1 mg/kg) in May 2002 by the Ministry of Health, Labour and Welfare (MHLW), the Ministry of Agriculture, Forestry and Fisheries (MAFF) added DON in imported wheat was added for the inspection program, and it was subjected to testing by trading companies with test results being released (245). On going surveillance of

mycotoxin contents in domestically produced wheat and barley have been conducted, with both DON and NIV being included in the test items (246). The MHLW has also conducted field surveillance on DON and NIV contamination through the Health and Labour Sciences Research programs, etc. The volume of domesticallyproduced and imported wheat grains in Japan are as shown in Table 19. Of all the wheat consumpted in Japan, approximately 85% is imported from US, Canada and Australia, and approximately 15% is produced in Japan

Table 19 Actual volume of domestic and imported wheat gains in Japan (in 10000 tons)

		FY 2002	FY 2003	FY 2004	FY 2005	FY 2006	FY 2007	FY 2008
Domestic production		83	86	86	88	84	91	88
Import	U.S.	230.3	286.0	275.7	257.7	272.6	294.5	294.2
	Canada	122.1	100.4	109.2	114.2	108.6	109.5	111.9
	Australia	87.6	119.8	112.9	106.8	114.8	85.3	79.9
	Others						0.3	0.3
Total import		440.0	506.1	497.9	478.7	496.0	489.6	486.3

FY: Fiscal year is 1st April through 31st March.

Summarized by the FSCJ based on the MAFF's "*Mugi no jukyū ni kansuru mitōshi* (Forecasts of wheat and barley supply and demand)" for fiscal 2007 and 2009 (247, 248).

(i) Results of surveys by the MAFF

a. DON

The surveillance results of DON in domestically produced wheat and inspection results of DON in imported wheat (at shipment) are shown in Table 20 and Table 21, respectively. The results of the surveillance and inspections of DON indicate that in both domestic and imported wheat, DON has been detected at levels above the quantification limits in some samples, however, none has been confirmed to contain DON beyond the temporary limit, except in fiscal 2002.

Table 20 Surveillance on deoxynivalenol (DON) in domestically produced wheat and barley grains (FY 2002 – 2007)

Item	FY	No. of items surveyed	Quantification limit (mg/kg)	No. of samples below quantification limit	%	Highest value (mg/kg)	Mean value (mg/kg) (i)	Mean value (mg/kg) (ii)	Mean value (mg/kg) (iii)
Wheat	2002	199	0.05	118	59%	2.1	0.16	-	-
	2003	213	0.05	136	64%	0.58	0.067	-	-
	2004	226	0.05	145	64%	0.93	0.044	-	-
	2005	200	0.010	128	64%	0.23	0.015	0.019	-
	2006	100	0.010	16	16%	0.88	-	-	0.13
	2007	100	0.009	43	43%	0.29	-	-	0.023
	2008	120	0.004-0.013	39	33%	0.46	-		0.033
Barley	2002	50	0.05	28	56%	4.8	0.26	-	-
	2003	54	0.05	34	63%	3.7	0.29	-	-
	2004	56	0.05	23	41%	1.8	0.24	-	-
	2005	50	0.010	23	46%	0.46	-	-	0.060
	2006	10	0.010	0	0%	2.5	-	-	0.55
	2007	10	0.007	3	30%	0.32	-	-	0.064

FY: Fiscal year is 1st April through 31st March.
Note 1: This table is taken from the Risk Profile Sheet on Food Safety (for review meeting) (249), with some modifications.
Note 2: The mean values for fiscal 2002 – 2004 were calculated based on the mean value (i).

For foscal 2005 onward, mean values were calculated based on the method proposed by the GEMS/Food (Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme)When more than 60% of all samples were below the quantification limit, mean values (i) and (ii) were used; and when 60% or less of all samples were below the quantification limit, mean value (iii) was used respectively based on the followings:

Mean value (i): All levels detected as below the quantification limit were substituted with "zero" in the calculation.

Mean value (ii): All levels detected as below the detection limit were substituted with detection limit and all levels at or above the detection limit and below the quantification limit were substituted with quantification limit in the calculation.

Mean value (iii): All levels detected as below the quantification limit were substituted with 50% of the quantification limit.

Table 21 Results of inspections on deoxynivalenol (DON) in imported wheat grains (at Ishipping)

	Quantification limit (mg/kg)	U.S.				Australia				Canada				France			
		No. of inspections	No. of detected cases	Detection rate	Range (mg/kg)	No. of inspections	No. of detected cases	Detection rate	Range (mg/kg)	No. of inspections	No. of detected cases	Detection rate	Range (mg/kg)	No. of inspections	No. of detected cases	Detection rate	Range (mg/kg)
FY 2002	–	84	19	0.23	0.05-0.68	33	0	0		40	7	0.18	0.07-0.28				
FY 2003	–	167	53	0.32	0.05-0.60	58	9	0.16	0.05-0.32	59	0	0					
FY 2004	0.05	168	77	0.46	0.05-0.71	51	0	0		63	1	0.02	0.07				
FY 2005	0.05	157	83	0.53	0.05-0.97	48	0	0		62	16	0.26	0.05-0.35				
FY 2006	0.05	162	94	0.58	0.05-1.00	53	0	0	0	59	22	0.37	0.06-0.38				
FY 2007	0.05	187	67	0.36	0.05-0.55	42	0	0		56	8	0.14	0.05-0.16	8	4	0.5	0.06-0.30
FY 2008	0.05*	187	59	0.32	0.05-0.62	62	12	0.19	0.08-0.31	55	24	0.44	0.06-0.31	6	2	0.33	0.2

FY: Fiscal year is 1st April through 31st March.

Note: This table was produced by the Food Safety Commission based on the results of the MAFF's inspections of residual pesticides, etc. in imported rice and wheat and barley (245).

*: The detection limit for the French data is 0.1 mg/kg.

The surveillance on DON in domestically produced wheat showed year-to-year variations, with the proportion of samples at or above the quantification limit ranging from 36 to 84% and the mean value from 0.015 to 0.16 mg/kg.

Similarly to the results of Japanese wheat, the inspections for DON in imported wheat showed year-to-year variations, with the detection rate ranging from 23 to 58%, 0 to 19%, and 0 to 44% in U.S., Australian and Canadian wheat, respectively, and the level of contamination ranging from 0.05 to 1.00 mg/kg, 0.05 to 0.32 mg/kg, and 0.05 to 0.38 mg/kg in U.S., Australian and Canadian wheat, respectively.

The surveillance for DON content in domestically produced barley showed year-to-year variations similarly to the results with the domestically produced wheat, with the proportion of samples at or above the quantification limit ranging from 37 to 100% and the mean value from 0.060 to 0.55 mg/kg (245, 246).

b. NIV

Table 22 shows the results of the surveillance on NIV.

NIV content has been surveyed together with DON as part of the surveillance on mycotoxins in domestically produced wheat. In wheat and barley, the proportion of samples at or above the quantification limit ranged from 32 to 70% and from 56 to 90%, respectively, with the mean value ranging from 0.010 to 0.087 mg/kg and from 0.042 to 0.58 mg/kg, respectively. Year-to-year variations are seen in NIV similary to DON (246).

Table 22 Surveillanceon on nivalenol (NIV) in domestically produced wheat and barley grains (FY 2002 – 2007)

Item	FY	No. of items surveyed	Quantification limit (mg/kg)	No. of samples below quantification limit	%	Highest value (mg/kg)	Mean value (mg/kg) (i)	Mean value (mg/kg) (ii)	Mean value (mg/kg) (iii)
Wheat	2002	199	0.05	130	65%	0.64	0.059	-	-
	2003	213	0.05	144	68%	0.55	0.040	-	-
	2004	226	0.024	118	52%	0.55	0.033	-	-
	2005	200	0.006	111	56%	0.20	-	-	0.010
	2006	100	0.007	30	30%	1.0	-	-	0.087
	2007	100	0.006	60	60%	0.21	-	-	0.013
	2008	120	0.005-0.013	66	55%	0.34			0.021
Barley	2002	50	0.05	22	44%	1.2	0.16	-	-
	2003	54	0.05	23	43%	0.95	0.13	-	-
	2004	56	0.024	14	25%	1.2	0.20	-	-
	2005	50	0.006	16	32%	0.38	-	-	0.042
	2006	10	0.007	1	10%	3.0	-	-	0.58
	2007	10	0.004	3	30%	0.33	-	-	0.051
	2008	100	0.009-0.014	45	45%	0.58	-	-	0.045

FY: Fiscal year is 1st April through 31st March.

Note 1: This table is taken from the Risk Profile Sheet on Food Safety (for review meeting) (250), with some modifications.

Note 2: The mean values for fiscal 2002 – 2004 were calculated based on the mean value (i).
For FY-2005 and onward, mean values were calculated, based on the method proposed by the GEMS/Food. When more than 60% of all samples were below the quantification limit, mean values (i) and (ii) were used; and when 60% or less of all samples were below the quantification limit, mean value (iii) was used, based on the followings:

Mean value (i): All levels detected as below the quantification limit were substituted with "zero" in the calculation.

Mean value (ii): All levels detected as below the detection limit were substituted with the detection limit and all levels at or above the detection limit and below the quantification limit were substituted with the quantification limit in the calculation.

Mean value (iii): All levels detected as below the quantification limit were substituted with 50% of the quantification limit.

The surveillance of contamination levels in domestic products showed no particular trend in terms of correlation between DON and NIV contamination.

(ii) Results of surveys by the MHLW

In FY 2001, a surveillance on DON and NIV contamination in wheat and barley was conducted as a special Health and Labour Sciences Research program.

The results are summarized in Table 23.

A total of 82 samples were tested, including 21, 36, 3 and 22 samples of imported wheat, domestic wheat, imported barley, and rye, respectively (detection limit: 0.001 mg/kg). The mean concentrations in data sets, in which toxin levels were above the quantification limit, were 238 µg/kg for DON and 10 µg/kg for NIV, with the ranges being 1 – 2,248 µg/kg and 1 – 110 µg/kg, respectively. Seventy-four percent of all samples showed co-contamination with both toxins (251).

In FY 2002, a surveillance on DON and NIV contamination in 124 samples of domestically produced brown rice was conducted as a special Health and Labour Sciences Research program. The results are summarized in Table 23. DON contamination was seen in 4 samples (range: 4.8 – 60.7 µg/kg; mean value of contaminated samples: 21.8 µg/kg; mean value of all samples: 4.8 µg/kg [weighted mean: 0.7 µg/kg]) and NIV contamination in 15 samples (range: 2.0 – 17.4 µg/kg; mean value of contaminated samples: 5.0 µg/kg; mean value of all samples: 6.7 µg/kg [weighted mean: 0.6 µg/kg]). Co-contamination with DON and

NIV was seen in 1 sample. It was demonstrated that after contaminated brown rice was polished, approximately 40% of DON and NIV in the brown rice remained in the polished rice (242).

In fiscal 2003, the MHLW conducted a field survey of DON and NIV contamination using 84 samples of household wheat flour (commercially available low-gluten flour, high-gluten flour, tempura flour, etc.) and 88 samples of baby food products (biscuits, curry roux, noodles, etc.), purchased in Hokkaido, Kanto, Osaka and Kyushu. The results are summarized in Table 23. In household wheat flour, the detection rates of DON and NIV were 80% and 31%, respectively, with the mean DON and NIV levels of 138 μg/kg (range: 5 – 1,147 μg/kg) and 81 μg/kg (range: 5 – 247 μg/kg), respectively. A correlation between DON and NIV contamination was seen in the samples of wheat flour purchased in Kyushu (14 of the 21 samples were local products), but not in terms of national average. The detection rate of DON in baby food products was 80%, with the mean level of 20 μg/kg (range: 2.5 – 59 μg/kg) (252).

Table 23 Surveillance on deoxynivalenol (DON) and nivalenol (NIV) in grains and foods prepared for infants

Fiscal year of survey (Ref.)	Sample	No. of samples	Mean value of contaminated samples (μg/kg)		Mean value of all samples (μg/kg)	
			DON	NIV	DON	NIV
FY 2001 (251)	Wheat (imported)	21	133.9 (1－740)	2.9 (1–7)	95.6*	1.2*
	Wheat (domestic)	36	358.4 (1－2248)	8.8 (1–27)	388.3*	8.3*
	Barley (imported)	3	9 (2－20)	5.5 (5–6)	9.0*	3.66*
	Rye (domestic)	22	8.1 (1－47)	15.1 (1–110)	6.2*	12.8*
FY 2002 (242)	Rice (domestic brown rice)	124	21.8 (4.8－60.7)	5.0 (2.0–17.4)	0.7****	0.6****
FY 2003 (252)	Household wheat flour	84	172.5**	89.8**	138 (5–1147)***	81 (5-247)***
	Baby/infant food products	88	20**	–	20 (2.5–59)***	–

FY: Fisical year is 1st April through 31st March.
Note: This table was composed by the Food Safety Commission based on the relevant reference materials.
*: Calculated by the FSCJ assuming ND as 0.
**: Calculated by the FSCJ using the following formula: (mean value of all samples) x (number of detected cases/number of samples).
***: Calculated by assuming ND as 5 mg/kg.
****: Calculated by assuming ND as 0.

In FY 2007, a surveillance on DON and NIV contamination was conducted as a Health and Labour Sciences Research program, using 59 samples of domestically produced wheat, excluding Hokkaido products, in order to estimate NIV exposure as described below. The results indicated that there is a relatively high correlation between DON and NIV contamination levels. As shown in Table 24, the numbers of samples whose content of DON, NIV and content both DON and NIV was at or below the detection limit were 6 (10.2%), 23 (39.0%) and 5 (8.5%) samples, respectively (253).

Table 24 Surveillance on deoxynivalenol (DON) and nivalenol (NIV) in domestically produced wheat (FY 2007; 59 samples in total)

mg/kg	DON	NIV	Total of both levels
<0.005	6	23	5
0.005≤	24	21	20
0.05≤	11	7	12
0.1≤	15	6	14
0.4≤	1	2	3
0.6≤	0	0	3
1.1≤	2	0	2

Fiscal year is 1st April through 31st March.
Note: This table is taken from the Health and Labour Sciences Research program, *Kabi doku wo fukumu shokuhin no anzensei ni kansuru kenkyū*, *Heisei 19 nendo, Sōkatsu/buntan kenkyū hōkokusho* (A Study on the Safety of Food Containing Mycotoxins, the FY 2007 Summary/ Grant Research Report) with some modifications (253).

(2) Estimation of exposure

Out of the main grains known to be contaminated with DON and NIV, rice and wheat are likely to be consumed in large amounts in Japan. With regards rice, there is a report using a provisional calculation of the amount of exposure based on the average contamination levels and average rice consumption, which reveals very low intakes of these toxins: 0.0029 μg/kg bw per day of DON and 0.0032 μg/kg bw per day of NIV in adults; as well as 0.0052 μg/kg bw per day of DON and 0.0056 μg/kg bw per day of NIV in infants aged 1 – 6 years (242). Since wheat is thus likely to be the main food contributing to DON and NIV intakes in Japan, DON and NIV exposures have been estimated based on data on consumption of foods containing wheat and data including surveillance of mycotoxin contents in these foods.

(i) A provisional calculation by total diet study (TDS) method

In FY 2005, the MHLW conducted a survey of intakes of DON and other trichothecene mycotoxins by total diet study (TDS) method using the market-basket method.[13] The survey of trichothecene mycotoxin contents in food groups I (rice and processed rice products), II (processed grain products and processed starch products), III (sugar and confectionery) and IV (preference beverages) in 4 regions found that food group II (processed grain products and processed starch products) was contaminated with DON in all 4 regions. Based on these results, the average DON intake per person in Japan was estimated, by assuming that the average consumption of foods from group II is 168.4 g based on the results of the FY 2002 national nutrition survey.

The results are shown in Table 25.

Table 25 Estimation of daily intake of deoxynivalenol (DON) by the total diet study method (FY 2005)

Food group	Region	DON level (μg/kg)	Consumption of food group (g)	DON intake	
				(ng/person)	(ng/kg bw/day)*
II (processed grain products, processed starch products)	Hokkaido	4.77	168.4	803.27	14.85
	Kanto	3.65	168.4	614.66	11.36
	Shikoku	4.10	168.4	690.44	12.76
	Kyushu	4.45	168.4	749.38	13.85

Fiscal year is 1st April through 31st March.
Note: This table was taken from the report of the survey on daily intakes of contaminants, etc. in foods (254) with some modifications.

[13] Total diet study (TDS) method: A wide range of food products are purchased at retail shops, etc. and are processed and prepared to make them ready for consumption, followed by an analysis to calculate the average chemical contents in each food group. By multiplying the resulting contents by a specific population's average consumption of each food group, the average chemical intakes can be estimated. There are the market-basket method and duplicate diet method.

*: The values with * are reproduced by the Food Safety Commission from the data in the report (i.e., the average body weight of an adult man and woman: 54.1 kg).

The estimated average intakes were 14.85, 11.36, 12.76 and 13.85 ng/kg bw per day in the Hokkaido, Kanto, Shikoku and Kyushu regions, respectively (254).

(ii) A provisional calculation using mean values

DON exposure was estimated by a special Health and Labour Sciences Research program conducted in FY 2002. Using the data on DON contamination levels obtained from the aforementioned MAFF's FY 2002 survey of imported and domestically produced wheat (0.16 and 0.06 mg/kg in domestic and imported wheat, respectively), the weighted mean DON levels were calculated based on the domestic supply of domestically produced and imported wheat in FY 1997 (540,000 and 4,560,000 tons of domestic and imported wheat, respectively). The average wheat consumption per person in Japan was based on the data from the FY 2000 national nutrition survey. The estimation also took into account the residue levels of DON estimated from an experiment on the reduction of DON during wheat processing, which was conducted in parallel with the aforementioned program. Based on the above data, the mean DON exposure was estimated.

The results are shown in Table 26.

Table 26 Estimation of daily exposure of deoxynivalenol (DON) using mean-DON contamination levels (FY 2002)

Age	Wheat consumption (g/day)	Daily intake (μg/kg bw/day)	Japanese person's body weight (kg)	Daily intake (μg/day/person)
Mean of all ages	94.3	0.13	52.6	6.70
Mean of age 1 – 6 years	64.1	0.29	15.9	4.55

FY: Fiscal year is 1st April through 31st March.
Note: This table was produced by the Food Safety Commission based on the report of the urgent research study for the establishment of standards for deoxynivalenol in wheat, etc. (257).

The estimated intakes were calculated to be 0.13 μg/kg bw per day (6.70 μg per day/person) and 0.29 μg/kg bw per day (4.55 μg per day/person) in terms of the mean of all ages and that of age 1 – 6 years, respectively (257).

In FY 2003, the MHLW conducted a surveillance on DON contamination in household wheat flour and wheat products for babies/infants. The daily DON intake was estimated based on the mean contamination levels obtained from this survey. The residue levels of DON in bread made from wheat and in noodles after preparation was set at 1 and 0.5, respectively. It was assumed that 50% of all wheat flour is consumed as bread and the remaining 50% as noodles.

The results are shown in Table 27.

Table 27 Estimation of daily exposure to deoxynivalenol (DON) by using mean DON contamination levels (FY 2003)

Age	Wheat consumption (g/day)	Daily intake (μg/kg bw/day)	Japanese person's body weight (kg)	Daily intake (μg/day/person)
Mean of all ages	98.0	0.17	52.6	8.8
Mean of age 1 – 6 years	64.1	0.36	15.9	5.7

Fiscal year is 1st April through 31st March.
Note: This table was produced by the Food Safety Commission based on the report of testing and inspection for mycotoxins in food (252).

The estimated intakes were calculated to be 0.17 μg/kg bw/day (8.8 μg per day/person) and 0.36 μg/kg bw per day (5.7 μg per day/person) as the mean of all ages and that of age 1 – 6 years, respectively (252).

(iii) A provisional calculation using a probabilistic approach

a. Estimation of DON exposure

From the 2002 national nutrition survey, food items containing wheat were selected and were classified into 5 categories (flours, breads, noodles, Chinese foods, and confectionery), followed by calculation of food consumption in each category based on the data from the survey. Then, in order to calculate the distribution of wheat consumption in each population, the wheat concentration in each category was set and a data set for simulation was constructed, assuming a log-normal distribution for each of the age strata (4 strata, including 1 – 6, 7 – 14, 15 – 19, and 20 ≤ year-old strata).

In addition, based on the results of the aforementioned MAFF's surveillance of DON content in domestically produced wheat from FY 2002 to 2004 and on the results of the MHLW's surveillance of DON contamination in FY 2003, DON exposure was estimated by a Monte Carlo simulation, by using the aforementioned data sets for simulation of the distribution of wheat consumption in the population, and by setting the following 3 scenarios for regulation of DON content in wheat (assuming a reduction rate of 50% in the milling process from unpolished wheat).

Scenario (i): No regulations.
Scenario (ii): 0.55 mg/kg as wheat flour (1.1 mg/kg as unpolished wheat)
Scenario (iii): 1 mg/kg as wheat flour (2.2 mg/kg as unpolished wheat)

The results are shown in Table 28.

No considerable difference was observed between the regulation scenarios. In the age strata, the 1 – 6 year-old stratum showed the highest exposure, with the 7 – 14 year-old and older strata showing similar values. In estimated exposure, none of the 95th percentile values of the strata was above 1 μg/kg bw per day, but the 99th percentile value was 2 – 3 μg/kg bw per day in the 1 – 6 year-old stratum and approximately 1 μg/kg bw per day in the 7 – 14 year-old and older strata.

Note that in the above results, a log-normal distribution of wheat consumption was assumed without setting the maximum value; instead, impractically high levels of wheat consumption were included in the distribution data sets. It is thus necessary to take into account the fact that the effect of this manipulation is greater particularly at higher percentiles (253).

In light of the trend for higher DON contamination levels in domestically produced wheat than in imported wheat in the FY-2002 survey, it is also necessary to note that the above estimation was based on the worst scenario which assumes that all wheat consumed was domestic products; and that there are large year-to-year variations in DON contamination levels, due to the effects of the climate, etc. of the year of wheat harvest (255).

Table 28 Estimation of daily exposure to deoxynivalenol (DON) in age groups by the monte-carlo simulation method

		Estimated exposure (μg/kg b.w./day)										
Age	Regulation	MIN	1%ile	5%ile	10%ile	25%ile	50%ile	75%ile	90%ile	95%ile	99%ile	MAX
1-6 years	None	0.00	0.00	0.00	0.01	0.03	0.08	0.19	0.48	0.85	2.58	772.53
	1.1 mg/kg	0.00	0.00	0.00	0.01	0.03	0.07	0.19	0.46	0.82	2.38	807.73
	2 mg/kg	0.00	0.00	0.00	0.01	0.03	0.08	0.19	0.47	0.85	2.54	915.47
7-14 years	None	0.00	0.00	0.00	0.00	0.01	0.04	0.08	0.20	0.36	0.97	513.98
	1.1 mg/kg	0.00	0.00	0.00	0.00	0.01	0.04	0.08	0.19	0.35	0.89	319.57
	2 mg/kg	0.00	0.00	0.00	0.00	0.01	0.04	0.08	0.20	0.36	0.95	1,092.02
15-19 years	None	0.00	0.00	0.00	0.00	0.01	0.03	0.08	0.19	0.36	1.08	3,357.92
	1.1 mg/kg	0.00	0.00	0.00	0.00	0.01	0.03	0.08	0.19	0.34	0.98	5,485.20
	2 mg/kg	0.00	0.00	0.00	0.00	0.01	0.03	0.08	0.19	0.35	1.06	3,929.46
20 ≤ years	None	0.00	0.00	0.00	0.00	0.01	0.03	0.08	0.18	0.32	0.94	32.66
	1.1 mg/kg	0.00	0.00	0.00	0.00	0.01	0.03	0.07	0.18	0.31	0.87	7.43
	2 mg/kg	0.00	0.00	0.00	0.00	0.01	0.03	0.08	0.18	0.32	0.93	11.07

Assumption B (for all samples below the detection limit, a uniform distribution between 0 and 0.05 mg/kg was used.)

		Estimated exposure (μg/kg b.w./day)										
Age	Regulation	MIN	1%ile	5%ile	10%ile	25%ile	50%ile	75%ile	90%ile	95%ile	99%ile	MAX
1-6 years	None	0.00	0.00	0.00	0.00	0.01	0.04	0.14	0.43	0.81	2.54	889.48
	1.1 mg/kg	0.00	0.00	0.00	0.00	0.01	0.04	0.14	0.41	0.77	2.33	917.10
	2 mg/kg	0.00	0.00	0.00	0.00	0.01	0.04	0.14	0.43	0.80	2.49	1,466.35
7-14 years	None	0.00	0.00	0.00	0.00	0.00	0.02	0.06	0.19	0.35	0.96	363.30
	1.1 mg/kg	0.00	0.00	0.00	0.00	0.00	0.02	0.06	0.19	0.34	0.88	243.03
	2 mg/kg	0.00	0.00	0.00	0.00	0.00	0.02	0.06	0.19	0.35	0.94	263.86
15-19 years	None	0.00	0.00	0.00	0.00	0.00	0.02	0.06	0.18	0.34	1.02	10,165.50
	1.1 mg/kg	0.00	0.00	0.00	0.00	0.00	0.02	0.06	0.18	0.33	0.92	5,416.47
	2 mg/kg	0.00	0.00	0.00	0.00	0.00	0.02	0.06	0.18	0.34	1.00	15,834.00
20 ≤ years	None	0.00	0.00	0.00	0.00	0.00	0.01	0.05	0.17	0.32	0.94	23.31
	1.1 mg/kg	0.00	0.00	0.00	0.00	0.00	0.01	0.05	0.16	0.31	0.87	11.43
	2 mg/kg	0.00	0.00	0.00	0.00	0.00	0.01	0.05	0.17	0.32	0.93	11.72

Note: Taken from the estimation of deoxynivalenol (DON) exposure to people in Japan through wheat consumption by Monte Carlo simulation (253) (with some modifications).

b. Estimation of NIV exposure

From the FY 2004 "Food Consumption and Consumption Frequency Survey," food items containing wheat were selected and were classified into 5 categories (a. flours, b. breads, c. noodles, d. Chinese foods, and e. confectionery). Then, in order to calculate the distribution of wheat consumption in each population, the wheat concentration in each category was set based on the same survey, etc. and a data set for simulation assuming a log-normal distribution was created for each of the age strata (4 strata, including 1 – 6, 7 – 14, 15 – 19, and 20 ≤ year-old strata).

Then, based on the results of the aforementioned surveillance of DON and NIV contamination in domestically produced wheat (excluding Hokkaido products) conducted by the Health and Labour Sciences

Research program in FY 2007 (253), NIV exposure was estimated by using data sets for simulation on distribution of wheat consumption in the population (setting a reduction rate of 50% in the milling process from unpolished wheat) , and by setting the following four scenarios for regulation of NIV under the current regulation of DON (unpolished wehat 1.1 mg/kg).

Under the current regulation of DON (unpolished wheat: 1.1 mg/kg),
◎Exposure to NIV was estimated using:
Scenario (i): No regulations on NIV
Scenario (ii): NIV (0.2 mg/kg) in wheat (unpolished)
Scenario (iii): NIV (0.5 mg/kg) in wheat (unpolished)
Scenario (iv): NIV (1.0 mg/kg) in wheat (unpolished)

The results are shown in Table 29.

Table 29 Estimation of daily exposure of different age groups to nivalenol (NIV) by the monte-carlo simulation method

1-6 years old

Scenario	50%ile	60%ile	70%ile	80%ile	90%ile	95%ile	97.5%ile	99%ile	99.5%ile	99.8%ile	99.9%ile
Regulations on NIV alone: None	0.01	0.02	0.05	0.09	0.19	0.33	0.52	0.85	1.17	1.71	2.20
Regulations on NIV alone: 0.2 mg/kg	0.01	0.02	0.04	0.08	0.16	0.26	0.39	0.61	0.81	1.13	1.42
Regulations on NIV alone: 0.5 mg/kg	0.01	0.02	0.05	0.09	0.19	0.33	0.51	0.83	1.13	1.63	2.09
Regulations on NIV alone: 1 mg/kg	0.01	0.02	0.05	0.09	0.19	0.33	0.52	0.85	1.17	1.70	2.21

7-14 years old

Scenario	50%ile	60%ile	70%ile	80%ile	90%ile	95%ile	97.5%ile	99%ile	99.5%ile	99.8%ile	99.9%ile
Regulations on NIV alone: None	0.01	0.02	0.03	0.07	0.14	0.23	0.36	0.58	0.79	1.13	1.44
Regulations on NIV alone: 0.2 mg/kg	0.01	0.02	0.03	0.06	0.11	0.19	0.27	0.41	0.53	0.72	0.89
Regulations on NIV alone: 0.5 mg/kg	0.01	0.02	0.03	0.07	0.14	0.23	0.35	0.56	0.76	1.07	1.35
Regulations on NIV alone: 1 mg/kg	0.01	0.02	0.03	0.07	0.14	0.23	0.36	0.58	0.79	1.12	1.44

15-19 years old

Scenario	50%ile	60%ile	70%ile	80%ile	90%ile	95%ile	97.5%ile	99%ile	99.5%ile	99.8%ile	99.9%ile
Regulations on NIV alone: None	0.01	0.01	0.03	0.05	0.11	0.18	0.28	0.44	0.59	0.83	1.04
Regulations on NIV alone: 0.2 mg/kg	0.01	0.01	0.02	0.05	0.09	0.15	0.21	0.31	0.39	0.52	0.63
Regulations on NIV alone: 0.5 mg/kg	0.01	0.01	0.03	0.05	0.11	0.18	0.27	0.43	0.57	0.79	0.98
Regulations on NIV alone: 1 mg/kg	0.01	0.01	0.03	0.05	0.11	0.18	0.28	0.44	0.59	0.83	1.04

20≤ years old

Scenario	50%ile	60%ile	70%ile	80%ile	90%ile	95%ile	97.5%ile	99%ile	99.5%ile	99.8%ile	99.9%ile
Regulations on NIV alone: None	0.00	0.01	0.02	0.03	0.07	0.11	0.17	0.28	0.37	0.53	0.67
Regulations on NIV alone: 0.2 mg/kg	0.00	0.01	0.02	0.03	0.06	0.09	0.13	0.19	0.25	0.34	0.41
Regulations on NIV alone: 0.5 mg/kg	0.00	0.01	0.02	0.03	0.07	0.11	0.17	0.27	0.36	0.50	0.63
Regulations on NIV alone: 1 mg/kg	0.00	0.01	0.02	0.03	0.07	0.11	0.17	0.28	0.37	0.53	0.67

Note 1: Extracted from the Health and Labour Sciences Research program, *Kabi doku wo fukumu shokuhin no anzensei ni kansuru kenkyū*, *Heisei 19 nendo, Sōkatsu/buntan kenkyū hōkokusho* (A Study on the Safety of Food Containing Mycotoxins, theFY 2007 Summary/Grant Research Report) (256) (with some modifications).
Note 2: Estimated exposure levels are in the units of μg/kg bw per day.

In theage strata, the 1 – 6 year-old stratum showed the highest exposure, with a trend for lower exposure in higher age strata. In estimated NIV exposure, none of the 95th percentile values of the strata was above 0.4 μg/kg bw per day, but the 99th percentile values were all above 0.7 μg/kg bw per day, except for the 1 – 6 year-old stratum under the scenario of a regulatory limit of NIV (0.2 mg/kg) alone (256).

Note that in the above results, a log-normal distribution of wheat consumption was assumed without setting the maximum value; instead, impractically high levels of wheat consumption were included in the distribution data sets. It is thus necessary to take into account the fact that the effect of this manipulation is greater particularly at higher percentiles. It is also necessary to note that: this estimation assumes that all wheat consumed was domestic products; that in the surveillance, the correlation between DON and NIV contamination may be found higher than it actually is, because samples did not include Hokkaido wheat, whose NIV contamination levels are relatively low and whose production volume is high; and that there are large year-to-year variations in DON and NIV contamination levels, due to the effects of the climate, etc. of the year of wheat harvest.

(3) Reduction during milling and cooking processes, etc.

The reduction rates of DON and NIV during milling were investigated using unpolished wheat and wheat flour milled from the same (previously-mentioned) unpolished wheat. For both unpolished wheat and milled wheat flour, 4 sets (for household consumption, confectionery, noodles and baking) of paired samples were used. A total of 160 samples were collected, 20 samples from each type. The mean DON and NIV contents in unpolished wheat were 184 μg/kg (range: 6 – 2452 μg/kg) and 23 μg/kg (7 – 174 μg/kg), respectively. The mean DON and NIV contents in wheat flour were 42.4 μg/kg (8 – 1620 μg/kg) and 3.41 μg/kg (4 – 20 μg/kg), respectively. The reduction rates during the milling process are shown in Table 30. The mean reduction rates were 73% and 57.7% for DON and NIV, respectively (257).

Table 30 Effects of milling ondeoxynivalenol (DON) and nivalenol (NIV) in wheat

		Overall	Type of wheat			
			Household	Confectionery	Noodles	Baking
Mean reduction rate of DON (%)	Mean±SE	73.0±2.70	69.4±5.75	78.9±5.31	74.0±6.75	72.6±4.61
Range of reduction rate (%)		25－97	38－92	43－94	25－94	29－97
No. of samples with detected DON after milling/no. of samples with detected DON before milling		59/77	18/20	11/20	11/17	19/20
Mean reduction rate of NIV (%)	Mean±SE	57.7±4.30	63.8±5.28	47.0±12.9	59.9±10.8	38.3±13.2
Range of reduction rate (%)		0-91	31－91	21－77	0－84	13－57
No. of samples with detected NIV after milling/no. of samples with detected NIV before milling		24/73	16/20	4/20	8/14	3/19

Note: This table was produced by the Food Safety Commission based on the report of the urgent research study for the establishment of standards for deoxynivalenol in wheat, etc. (257).

In these results, the levels detected in samples as below the detection limit after milling was not calculated, and data on these samples were not summarized. It should be noted that for NIV levels detected in high proportions of samples were below the detection limit after milling, and that the contamination levels in pre-milled wheat were relatively low.

A study on the reduction of DON during milling and cooking was conducted by a Health and Labour Sciences Research program. Contaminated unpolished wheat was milled and measured for DON levels. Then, DON-contaminated high-gluten flour and noodle wheat flour were prepared and measured for DON levels after the former was processed, made into bread and steamed buns, and the latter processed and cooked into UDON noodles. The mean reduction rates during milling were 61.3% and 49.5% in unpolished wheat with DON levels 0.78 μg/kg and 0.20 μg/kg, respectively. The reduction rates during baking,

cooking and steaming were 0.12%, 71.1% and 17.9% in bread, UDON noodles and steamed buns, respectively. Since DON is water-soluble, it was likely that DON in UDON noodles was reduced effectively by transferring into cooking water (257).

Table 31 Reduction of deoxynivalenol (DON) by milling and cooking using a home cooking machine

Reduction rate during milling (%)	Reduction rate during cooking (%)	
61.3% (unpolished wheat, 0.78 µg/kg) 49.5% (unpolished wheat, 0.20 µg/kg)	**Bread**	0.12
	UDON noodles	71.1
	Steamed buns	17.9

Note: This table was produced by the Food Safety Commission based on the report of the urgent research study for the establishment of standards for deoxynivalenol in wheat, etc. (257).

The reduction of DON during processing and cooking UDON noodles as well as processing, baking and steaming bread from wheat flour using household applicances was compared between HPLC and bioactivity analysis. The WST-8 and BrdU assays using 3T3 cells were employed for the bioactivity analysis..

The results are shown in Table 32.

Table 32 Residual deoxynivalenol (DON) retention at each processing stage of UDON noodles and bread measured by HPLC and cytotoxicity assay

A. UDON noodles (made by using household noodle making machines)

	HPLC (residual rate in %)	Bioactivity analysis	
		WST-8 (residual rate in %)	BrdU (residual rate in %)
Wheat flour	100.29±3.65	100.29±3.65	100.29±8.78
Udon noodles before boiling	98.55±4.08	98.55±4.08	98.84±6.78
Udon noodles after boiling	30.52±4.08	34.53±1.29	28.88±5.02
Water used for boiling	41.28±3.89	64.97±3.99	42.89±4.58

B. Bread (made by using household bread making machines)

	HPLC (residual rate in %)	Bioactivity analysis	
		WST-8 (residual rate in %)	BrdU (residual rate in %)
Wheat flour	100.00±7.04	100.00±4.10	100.00±1.53
Bread	108.42±8.45	84.05±4.34*	92.30±1.03*

*: Significantly different from the corresponding HPLC results at $p<0.05$.
Taken from literature (258) (translated into Japanese with some modifications).

In UDON noodles, an approximately 70% reduction of DON was observed during cooking by both the HPLC and bioactivity analysis, with no significant differences between the both analysis. In bread, on the other hand, no reduction was observed by HPLC, while the bioactivity analysis indicated a reduction, with significant differences between the HPLC and bioactivity analysis. This could be due to DON complex formation during bread making, which can be attributable to weakend toxicity of DON (258).

Reduction of DON and NIV during commercial-scale baking was investigated, using paired samples consisting of bread products produced at bread plants in 9 different regions in Japan and wheat flours used as ingredients in the preparation of the above bread products. The investigation of 35 samples of bread and 35 samples of wheat flour (a total of 70 samples) for DON and NIV contamination revealed mean DON and NIV reduction rates of 25.6% and 34.2%, respectively, during commercial-scale baking (Table 33).

Table 33 Mean reduction rates of deoxynivalenol (DON) and nivalenol (NIV) during bread making (in commercial scale)

	DON	NIV
Reduction rate during bread making (in commercial scale)	25.6%	34.2%

(259)

Potential reasons for the considerable differences between reduction rates during the baking process using commercial-scale machines and that by using household baking machines are as follows : (i) reduction may have different trends depending on the scale of baking process, i.e. while household bread making machines were used in the baking process described earlier, the latter-described baking process was on a large, commercial scale; and (ii) lower levels of contamination may be associated with higher reduction rates.

Decomposition by roasting of DON and NIV in naturally contaminated barley was analyzed by GC-MS or ELISA using monoclonal antibodies. The GC-MS analysis confirmed that decomposition of DON and NIV is dependent on the heating temperature and heating time. However, after heating for 5 or 30 min at 150ºC, the GC-MS analysis indicated a slight reduction, while the ELISA conversely showed an increase. These results suggest that thermal decomposition products of DON and NIV have high levels of cross reactivity with the monoclonal antibodies (260).

Reduction of DON during the processes from milling to cooking was investigated using durum wheat spaghetti. When the DON content in unpolished wheat was taken as 100%, the DON residue rates after milling, noodle making (before cooking) and cooking were 36.5 ± 12.9%, 32.6 ± 12.3% and 19.5 ± 7.8%, respectively (261). These results indicate that the reduction rate of DON in durum wheat spaghetti during cooking is approximately 40%.

DON is known to increase during the process of fermentation. It has been reported that there was little reduction of DON in yeast-fermented bread products (262, 263, 264, 265, 266) and that, on the contrary, DON increases during yeast fermentation (267). Studies on brewing suggest that the increase of DON during this type of processing is caused by conversion of DON precursors and DON complexes contained in the raw materials to DON (268, 269).

Many other studies have been conducted on reduction of DON during milling and cooking. Reports of these studies indicate that DON reduces during milling but cannot be eliminated completely by normal cooking processes due to its heat resistance. They do indicate, however, that due to its high solubility in water DON transfers easily into water during cooking in boiling water (270).

IV. Risk Assessment

A risk assessment of deoxynivalenol and nivalenol was conducted using the materials listed in the references section. In setting tolerable daily intakes (TDIs), the assessment was conducted based on studies involving administration of purified toxins.

1. Deoxynivalenol (DON)

After oral administration, DON mainly undergoes de-epoxydation by the intestinal flora in the gastrointestinal tract, as well as glucuronate conjugation *in vivo*, thereby being converted and metabolized to less toxic derivatives, followed by excretion in urine and stool together with unchanged DON.

Major findings from toxicity studies using experimental animals include emesis, decreased feed consumption, suppressed body weight gain and effects on the immune system. At doses higher than those at which the above effects were found, fetal toxicity and teratogenicity were found.

In genotoxicity studies, while positive results were obtained in some chromosome aberration tests, etc., the toxicity was not high. In addition, DON showed no carcinogenicity in a 2-year chronic toxicity study using mice. From these results, it was considered unlikely that DON has *in vivo* genotoxic potential. The IARC has classified toxins produced by *Fusarium sp.*, including DON, as Group 3, i.e., not classifiable as to carcinogenicity to humans.

Based on the above reasons, the Commission has concluded that at this point, DON cannot be regarded as genotoxic or carcinogenic, and that a TDI can be set for it.

The following points were taken into account in setting a TDI.

Of the findings obtained in the various toxicity studies, emesis was seen at quite low doses (i.e., 0.05 – 0.1 mg/kg bw) in single oral dose studies in pigs. However, these results were obtained after treatment by oral gavage (solvent: water or saline), and after in-feed treatment, no emesis was found even at higher doses (0.19 – 0.6 mg/kg bw). The Commission decided to take into account the results obtained after in-feed treatment, since in-feed treatment is closer to the way people ingest the toxin from foods than treatment by oral gavage.

Of the effects on the immune system, resistance to infection was found to be weakend, as decreased survival rates after infection with *S. enteritidis*, was seen in the 0.12 mg/kg bw/day and higher dose groups, in a study using mice. However, the Commission considered it inappropriate to use the results of this study as a basis for setting a TDI, since the doses at which resistance to infection was observed were higher than the NOAEL obtained in the 2-year chronic toxicity study using mice, and since the reaction used as the index in this test system included potential effects of the pathogen.

In a study using pigs, in-feed treatment with DON resulted in a dose-dependent decrease in the secondary antibody response to tetanus toxin. However, the Commission considered it inappropriate to use the results of this study as a basis for setting a TDI, since the study used naturally contaminated feed instead of purified DON, and since the no effect dose cannot be determined due to the lack of a control group of animals fed a diet without treatment with the toxin.

With respect to effects on blood IgA, a study using mice showed increased blood IgA levels after treatment with 0.071 mg/kg bw of DON by oral gavage 3 days per week for a 4-week period. However, the Commission decided not to use these results as a basis for setting a TDI, considering that: there was no dose relationship and the increase was slight; that no other feeding study using mice has shown any effects at such a low dose; and that the 2-year chronic toxicity study in mice showed no IgA accumulation in renal mesangial cells and no nephropathy.

Therefore, the Commission considered that adequate safety is ensured by setting the NOAEL at 0.1 mg/kg bw per day based on the suppression of body weight gain observed in the 2-year chronic toxicity study in mice and by setting a TDI based on this NOAEL.

For the above reasons, the TDI for DON was set at 1 μg/kg bw per day, by applying an uncertainty factor of 100 (10 for inter-species differences and 10 for inter-individual variations) to the above NOAEL of 0.1 mg/kg bw per day.

2. Nivalenol (NIV)

After oral administration, NIV mainly undergoes de-epoxydation by the intestinal flora in the gastrointestinal tract, thereby being converted and metabolized to less toxic derivatives, followed by excretion in urine and stool together with unchanged NIV.

Major findings from toxicity studies using experimental animals include decreased feed consumption, suppressed body weight gain and effects on the immune system. At doses higher than those at which the above effects were found, embryotoxicity was found.

In genotoxicity studies, the toxicity levels were not considered high though positive results were obtained in some chromosome aberration tests. While positive results were obtained in some comet assays, a study on potential mutations in transgenic mice gave negative results. This result suggest that NIV causes initial damage to genes, and that this damage can be repaired, making mutations unstable. It should be noted however that the paucity of existing data made it difficult to assess the genotoxic potential of NIV at this time. As a matter of fact, a 2-year chronic toxicity study using mice indicated no carcinogenicity, and in a medium-term hepatocarcinogenicity study using rats, no changes in GST-P-positive foci were observed in the group treated with NIV alone or the group treated with NIV and DEN. However, the group subjected to initiation by DEN followed by treatment with AFB1 and then with NIV showed an increase in the area of GST-P-positive foci compared with the group treated with AFB1 alone after initiation by DEN, suggesting that NIV enhanced the induction of liver cancer by AFB1 after initiation by DEN. The IARC has classified toxins produced by *Fusarium sp.*, including NIV, as Group 3, i.e., not classifiable as to carcinogenicity to humans.

For the above reasons, the Commission concluded that a TDI can be set for NIV, considering that: while NIV enhanced the induction of liver cancer in rats by AFB1 after initiation by DEN, no carcinogenesis-promoting effect was observed after treatment with NIV alone following initiation by DEN; and that no carcinogenicity was found in the 2-year chronic toxicity study using mice.

Of the various toxicity studies, a study investigating effects on the immune system using mice showed increased blood IgA levels after treatment with NIV (0.071 mg/kg bw) by oral gavage 3 days per week for a 4-week period. However, the Commission decided not to use these results as a basis for setting a TDI, considering that: there was no clear dose relationship and the increase was slight; that no other feeding study using mice has shown any effects at such a low dose; and that 1-year and 2-year chronic toxicity studies in mice showed no histological changes in the kidneys and no nephropathy.

The Commission considered that adequate safety is ensured by setting the LOAEL at 0.4 mg/kg bw/day based on decreased WBC counts observed in sub-acute toxicity study in rats with 90 day oral administration and by setting a TDI based on this LOAEL.

For the above reasons, the TDI for NIV was set at 0.4 μg/kg bw/day, by applying an uncertainty factor of 1,000 (10 for interspecies differences and 10 for inter-individual variations, and 10 for the adopted LOAEL value derived from the subacute toxicity study) to the above LOAEL of 0.4 mg/kg bw per day.

3. Establishment of a group TDI for DON and NIV

Establisment of a group TDI for DON and NIV was considered difficult at present, due to the limited number of studies and varied test results on the combined effects of the two toxins, and the fact that the mechanism of action of each toxin has not been fully clarified. However, since it is inferred from their very similar chemical structures that DON and NIV are likely to have similar toxic effects, the Commission considers it desirable to hold discussions for setting a group TDI once relevant information is obtained.

4. Status of exposure

Although no detailed analysis has been conducted on the contribution of different foods to DON and NIV exposures in Japan, foods containing wheat are estimated to be the main source of exposure from the current situation of food contamination and food consumption.

An investigation of DON and NIV intakes by TDS method revealed that the average exposure to DON was 11.36 – 14.85 ng/kg bw/day. On the other hand, exposure to NIV could not be estimated since the toxin was not detected in any of the samples.

Based on the average mycotoxin contamination level found in surveillance of wheat and on the averege wheat consumption of people in Japan, the estimated exposure to DON was 0.13 – 0.17 μg/kg bw per day

and 0.29 – 0.36 μg/kg bw per day on average of all ages and that of 1 – 6 years, respectively.

Exposures to DON and NIV were estimated using a probabilistic approach based on the results of surveillance of DON and NIV contaminations in domestically produced wheat and on the consumption of foods containing wheat. The results showed that in all age groups, the 95th percentile exposures to DON and NIV were less than 1 μg/kg bw per day and less than 0.4 μg/kg bw per day, respectively. However, this estimation assumed the reduction rates of both DON and NIV during milling from unpolished wheat at 50% based on experiments, without considering reductions during other processing and cooking processes. Therefore, the actual exposures are likely lower than these estimations. It must also be noted that in the above results, a log-normal distribution of wheat consumption was assumed without setting the maximum value; instead, impractically high levels of wheat consumption were included in the distribution data sets. It is thus necessary to take into account the fact that the effect of this manipulation is greater particularly at higher percentiles. Furthermore, attention must be paid to the fact that the above provisional calculation was based solely on the results of surveillance of DON and NIV contamination in domestically produced wheat and did not take into account the contamination status in imported wheat, and that the results have uncertainty due to the wide variations in mycotoxin contamination levels because of the effects of the climate, etc. of the year of wheat harvest.

5. Summary

<Deoxynivalenol (DON)>
TDI: 1 μg/kg bw/day

(Rationale for TDI setting)	A chronic toxicity study
(Animal species)	Mouse
(Period)	2 years
(Method of administration)	Diet
(Finding providing rationale for NOAEL)	Suppressed body weight gain
(NOAEL)	0.1 mg/kg bw/day
(Uncertainty factor)	100 (10 each for interspecies and individual variations)

<Nivalenol (NIV)>
TDI: 0.4 μg/kg bw/day

(Rationale for TDI setting)	A subacute toxicity study
(Animal species)	Rat
(Period)	90 days
(Method of administration)	Diet
(Finding providing rationale for LOAEL)	Decrease in WBC count (females)
(LOAEL)	0.4 mg/kg bw/day
(Uncertainty factor)	1,000 (10 each for interspecies and individual variations, and 10 for the adoption of the LOAEL found in the subacute toxicity study)

Estimates of exposure to DON and NIV in Japan were considered below the established TDIs (1 μg/kg bw per day for DON; and 0.4 μg/kg bw per day for NIV). Therefore, dietary intake of DON and NIV was considered unlikely to cause adverse effects on health in the general population of Japan.

A provisional limit of 1.1 mg/kg has been set for DON in unpolished wheat, and measures to reduce DON and NIV contamination in production processes have been taken. However, followings should be considered: the estimation of exposures using a probabilistic approach revealed estimated values that were relatively close to the TDIs, particularly in children, and that there are wide variations in mycotoxin contamination due to the effects of climate, etc. of the year of harvest. Therefor, the Commission deems it desirable to steadily promote the measures currently taken to reduce DON and NIV contamination in production processes, as well as to discuss the necessity for setting standard limits for these toxins.

6. Future tasks

During the discussions on the present risk assessment of DON and NIV, the following information including data, etc. were regarded necessary to further improve the Commission's risk assessment:

- Information on the safety of DON and NIV analogues (acetylated, glycosidic and other analogues)
- Information on genotoxicity (particularly of NIV)
- Information on chronic toxicity and carcinogenicity in animal species other than mouse
- Information on combined effects of trichothecenes, including DON and NIV
- Human epidemiological data
- Data on actual DON and NIV (including acetylated, glycosidic and other analogues) contamination
- Discussion on use of the benchmark dose method in setting TDIs

<List of abbreviations of test items, etc.>

Abbreviation	Full name
15-AcDON	15-acetyldeoxynivalenol
3-AcDON	3-acetyldeoxynivalenol
AcDON	Acetyldeoxynivalenol
4-AcNIV	4-acetylnivalenol (fusarenon-X)
$5HT_3$	5-hydroxytryptamine (serotonin)
AFB1	Aflatoxin B_1
Akt	Serine/threonine protein kinase
ALT	Alanine transaminase
AP-1	Activator protein 1
ASAT	Aspartate aminotransferase
AST	Alanine aminotransferase
AUC	Area under the blood concentration curve
Bax	Bcl2-associated X protein
BMD	Benchmark dose
BrdU	5-bromo-2'-deoxyuridine
cAMP	Cyclic adenosine monophosphate
CD	Cluster of differentiation (The term "CD" followed by a number is used to name a surface antigen. Combinations of expressed CD antigens are analyzed or otherwise used to classify cells or analyze their functions, etc.)
CFU-GM	Colony forming units for granulocytes and macrophages
CINC	Cytokine-induced neutrophil chemoattractant
CnAβ	Calmodulin-dependent protein phosphatase Aβ
COX-2	Cyclooxygenase-2
CREB	cAMP response element binding protein
CYP	Cytochrome P450
DCNB	Dichloronitrobenzene
DAS	Diacetoxyscirpenol
DEN	Diethyl(nitroso)amine
DHA	Docosahexaenoic acid
DMSO	Dimethyl sulfoxide
DNA	Deoxyribo nucleic acid
DON	Deoxynivalenol
ED_{50}	50% effective dose
ELISA	Enzyme-linked immunosorbent assay
EPK	Extracellular signal-regulated kinase
FB1	Fumonisin B_1
Fra-2	Fos-related antigen-2
FSH	Follicle stimulating hormone
GC	Gas chromatography

GEMS/Food	Global Environment Monitoring System/Food Contamination Monitoring and Assessment Programme
GGT	Gamma-glutamyl transferase (gamma-glutamyl transpeptidase [γ-GTP])
GM	Granulocyte-monocyte
GST-P	Placental glutathione S-transferase
HPLC	High performance liquid chromatography
HPRT	Hypoxanthine-guanine phosphoribosyltransferase
IC_{50}	50% inhibitory concentration
IFN	Interferon
Ig	Immunoglobulin
IGF1	Insulin-like growth factor 1
IGFALS	Insulin-like growth factor binding protein, acid labile subunit
IL	Interleukin
iNOS	Inducible nitric oxide synthase
JNK	c-Jun N-terminal kinase
LD_{50}	50% lethal dose
LH	Luteinizing hormone
LPS	Lipopolysaccharide
MCP	Monocyte chemotactic protein
MHC	Major histocompatibility complex
MIP	Macrophage inhibitory protein
MKP1	Mitogen-activated protein kinase phosphatase-1
mRNA	Messenger RNA (ribonucleic acid)
MS	Mass spectrometry
Msk1	Mitogen- and stress-activated protein kinase 1
MTS	3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium
MTT	3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide
NF-κB	Nuclear factor kappa B
NIV	Nivalenol
NK	Natural killer
NO	Nitric oxide
OVA	Ovalbumin
PARP	Poly(ADP-ribose)polymerase
PHA	Phytohemagglutinin
PKR	Polyketide reductase
PM-TDI	Provisional maximum tolerable daily intake
PW	Pokeweed
RIA	Radioimmunoassay
RNA	Ribonucleic acid
RSK1	p90 ribosomal S6 kinase 1

SCE	Sister chromatid exchange
SCF	European Commission Scientific Committee on Food
SOCS	Suppressor of cytokine signaling
TDI	Tolerable daily intake
TDS	Total diet study
TEER	Transepithelial electrical resistance
TLC	Thin layer chromatography
TNF	Tumor necrosis factor
tTDI	Temporary tolerable daily intake
UDS	Unscheduled DNA synthesis
WST-8	2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt
ZEN	Zearalenon
α-ZEA	α-zearalenol

<Attached Table>

Results of toxicity studies using unpurified deoxynivalenol (DON)

Description of Target (Number per group)	Rout	Administered material	Administration period	Dose (mg/kg feed)	Dose (mg/kg bw/day)	Effects	LOAEL (mg/kg bw/day)	NOAEL (mg/kg bw/day)	Reference
Rat, Wistar, 139 g (5 females/ group)	Ddiet	Contaminated corn	8 days	0, 40	0, 2*	- Decreased feed consumption and weight gain, decreased absolute weights of liver and thymus, and increased hemoglobin, hematocrit and serum parameter values.	2*		271
	Diet	Contaminated corn detoxified by sodium bisulfate and autoclaving	8 days	0, 40	0, 2*	- Decreased serum alkaline phosphatase activity.	2*		
Rat, Sprague-Dawley, males 190-210 g, females165 g (10 males + 25 females/ group)	Diet	Artificially contaminated corn (*Fusarium graminearum NRRL 58839,* 96% DON, the remaining 4% was 3,15-dihydroxy-12, 13-epoxytrichothec-9-ene-8-one; no other trichothecenes or ZEN detected)	60 and 15 days in males and females, respectively, before mating	0, 20	0, 2*	- Decreased feed consumption, weight gain and fertility.	2*		134
Pig, young, 7.1-8.4 kg (2-4/ group)	Diet	Artificially contaminated corn (containing 875 mg/kg of DON, 3.9 mg/kg of ZEN; no T-2 toxin, DAS or 4-AcNIV detected)	21 days	0, 1.3, 12, 20, 43	0, 0.06, 0.6, 0.8, 1.6*	- Decreased feed consumption and weight gain.	0.06*		107
Pig, 8 kg (4 each sex/ group)	Diet	Contaminated wheat (only DON was quantified)	21 days	0, 0.9, 2.0, 2.8	0, 0.09, 0.18, 0.25*	- Decreased feed consumption and weight gain.	0.18*	0.09*	110
Pig, 60.5 kg (2 each sex/ group)	diet	Contaminated wheat (only DON was quantified)	42 days	0, 0.9, 2.2, 2.8, 4.2	0, 0.04, 0.09, 0.11, 0.17*	- Decreased feed consumption and weight gain.	0.09*	0.04*	
Pig, 7-week-old, 13.6 kg (6 neutered males/ group)	Diet	Contaminated wheat (containing 27 mg/kg of DON)	28 days	0, 4.5	0, 0.2*	- Renal lesions: after co-administration with FB1, decreased feed consumption and weight gain.	0.2*		272
Pig, Yorkshire, 6-7-week-old, 13 kg (6-8 neutered males/ group)	Diet	Naturally contaminated corn (containing 28.7 mg/kg of DON, 8.6 mg/kg of 15-AcDON and 1.1 mg/kg of ZEN)	28 days	0, 0.95, 1.78, 2.85	0, 0.08, 0.13, 0.18*	- Decreased weight gain. - Decreased thyroid weight. - Increased thyroxine, serum albumin and A/G ratio. - Decreased α-globulin.	0.13*	0.08*	273
Pig, Yorkshire, 10-13 kg (6 neutered males/ group)	Diet	DON-contaminated corn (containing 38.5 mg/kg of DON, 3.0 mg/kg of 15-AcDON and 1.3 mg/kg of NIV)	32 days	0, 1, 3	0, 0.09, 0.22*	- Suppressed weight gain. - Decreased serum α-globulin. - Increased cortisol.	0.22*	0.09*	122

Description of Target (Number per group)	Rout	Administered material	Administration period	Dose (mg/kg feed)	Dose (mg/kg bw/day)	Effects	LOAEL (mg/kg bw/day)	NOAEL (mg/kg bw/day)	Ref.erence
Pig, 12-13-week-old, 38 kg (6/ group)	Diet	Artificially contaminated corn (containing 2.5 mg/kg of DON; infected with *F. graminearum Schwabe DAOM180377*)	35 days	0, 2.5	0, 0.1*	- Decreased feed consumption and weight gain.	0.1*		274
Pig, Yorkshire, 18 kg (8 neutered male/ group)	Diet	Naturally contaminated corn (containing 28.7 mg/kg of DON, 8.6 mg/kg of 15-AcDON and 1.1 mg/kg of ZEN)	42 days	0, 4	Starting at 0.26, ending at 0.16*	- Decreased weight gain and feed consumption. - More corrugated stomach. - Decreased serum proteins.	0.26*		275
Pig, Norwegian Landrace, 59-day-old, 21 kg (7-11 each of females and neutered males/ group)	Diet	Naturally contaminated oats (containing 12.4 mg/kg of DON, 1.5 mg/kg of 3-AcDON, trace amounts of NIV and FUS-X, and 0.75 mg/kg of ZEN)	95 days	0, 0.7, 1.7, 3.5	0, 0.04, 0.1, 0.2*	- Decreased feed consumption and weight gain, increased liver weight, and decreased serum albumin.	0.1*	0.04*	177
Pig, Norwegian Landrace, 25 kg (5-9 females and 2-8 neutered males/ group)	Diet	Naturally contaminated oats (containing 14.6 mg/kg of DON, 1.76 mg/kg of 3-AcDON, and trace amounts of NIV and ZEN)	100 days	0, 0.5, 1, 2, 4	0, 0.02, 0.04, 0.08, 0.16*	- Decreased weight gain and feed consumption.	0.16*	0.08*	276
Pig (6/ group)	Diet	Naturally contaminated	5-11 weeks	0, 3.5- 4.4	0, 0.083 - 0.213	- The phagocytic ability of isolated monocyte-derived macrophages was lower in the DON-treated group. - No changes in T-cell stimulating ability.			277
Horse, 12.5-year old, 444 kg (5 each sex/ group)	Diet	Naturally contaminated barley (containing 36- 44 mg/kg of DON)	40 days		0.11*	- No effects on feed consumption, weight gain or serm parameters		0.11*	278
Cattle, holstein, in early lactation (2 females/ group)	Diet	Contaminated barley (containing 24 mg/kg of DON)	21 days	0, 2.1, 6.3, 8.5	0, 0.075, 0.22, 0.3	- No effects on feed consumption, weight gain, rumen pH or milk yield.		0.3	279
Cattle, neutered calves, 293 kg (18 males/ group)	Diet	Artificially contaminated barley (containing 22.2 mg/kg of DON)	84 days	0.9, 3.7, 6.4, 9.2	0.01, 0.05, 0.07, 0.1*	- No effects on feed consumption, weight gain or serum parameters.		0.1*	280
Lamb, 3-6-month old, 18 kg (3-4 each sex/ group)	Diet	Naturally contaminated wheat (containing 26 mg/kg of DON; ZEN was not detected)	28 days	0, 15.6	0, 0.94*	- No effects on feed consumption, weight gain, or hematological, serological and histological parameters.		0.94*	281
Broiler chick, 1-day-old (36 males/	Diet	Naturally contaminated wheat (containing 27 mg/kg of DON;	21 days	0, 16	0, 1.5*	- No effects on feed consumption, weight gain, or hematological, serological or histological		1.5*	282

Description of Target (Number per group)	Rout	Administered material	Administration period	Dose (mg/kg feed)	Dose (mg/kg bw/day)	Effects	LOAEL (mg/kg bw/day)	NOAEL (mg/kg bw/day)	Ref.erence
group)		aflatoxin, ZEN, ochratoxin, cyclopiazonic acid, moniliformin and fumonisin were below the detection limits)				parameters.			
Broiler chick, 1-day-old (60 males/ group)	Diet	Naturally contaminated wheat (containing 26 mg/kg of DON; ZEN, T-2 toxin, diacetoxyscirpenol, aflatoxin and ochratoxin were not detected)	21 days	0, 16	0, 1.3*	- Decreased feed efficiency.	1.3*		283
Broiler chick, 1-day-old (36 males/ group)	Diet	Naturally contaminated wheat (containing 27 mg/kg of DON; ZEN was not detected)	21 days	0, 15	0, 1.3*	- No effects on feed consumption, weight gain, or hematological or serological parameters. - Increased relative weights of the heart, bursa of Fabricius and gizzard.	1.3*		284
Broiler chick, 1-day-old (240 each sex/ group)	Diet	Naturally contaminated oats (containing 12.1 mg/kg of DON, 1.8 mg/kg of 3-AcDON and 1.4 mg/kg of ZEN)	35 days	0.1, 1.0, 2.1, 3.4 (containing 0, 0.18, 0.3, 0.53 of 3-AcDON, respectively, and 0, 0.15, 0.26, 0.5 of ZEN, respectively)	0.01, 0.1, 0.21, 0.34*	- No effects on feed consumption, weight gain, carcass weight, or heart or histological parameters.		0.34*	285
Broiler chick, 1-day-old (45/ group)	Diet	Contaminated corn (containing 9.8 mg/kg of DON, 1.24 mg/kg of 15-AcDON, 0.725 mg/kg of NIV, 1.15 mg/kg of ZEN, 1.04 mg/kg of moniliformin, 1.43 mg/kg of beauvericin and 0.105 mg/kg of FB1)	37 days	1.8, 3.6, 5.3 + 50% of other mycotoxins	0.14, 0.3, 0.46*	- No effects on weight gain, feed conversion rate or serological parameters. - At the highest dose, significant increase in heart weight.	0.46*	0.3*	286
Mallard, 1-year-old (10 each sex/ group)	Diet	Naturally contaminated wheat	14 days	0, 5.8	0, 1.5*	- No effects on serological, hematological or histological parameters.		1.5*	287
Dog, Beagle or Brittany, 1-7-year-old, 15-20 kg (2-14/ group)	Diet	Naturally contaminated wheat (containing 37 mg/kg of DON and 1 mg/kg of 15-AcDON)	14 days	0, 1, 2, 4, 6, 8, 10	0, 0.075, 0.15, 0.3, 0.45, 0.6, 0.75*	- Emesis and decreased feed consumption.	0.45*	0.3*	112
Cat, American Shorthair, 1-9-year-old, 1-4 kg (2-8/group)	Diet	Naturally contaminated wheat (containing 37 mg/kg of DON and 1 mg/kg of 15-AcDON)	14 days	0, 1, 2, 4, 6, 8, 10	0, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5*	- Emesis and decreased feed consumption.	0.4*	0.3*	112

*: Converted values based on the JECFA standards.

<References>

1 Worldwide regulations for mycotoxins in food and feed in 2003: FAO Food and Nutrition Paper 81.

2 EC Commision Regulation No.1126/2007. (http://www.fsai.ie/uploadedFiles/Commission_Regulation_EC_No_1126_2007.pdf)

3 JECFA monographs DEOXYNIVALENOL: Evaluation of certain mycotoxins. WHO Food Additives Series, No.47/FAO Food and Nutrition Paper 74, 2001.

4 IARC Monographs on the evaluation of carcinogenic risks to humans; Vol.56: Some Naturally Occurring Substances: Food Items and Constituents, Heterocyclic Aromatic Amines and Mycotoxins, 1993, 397-444.

5 The MERCK INDEX, 13th, Publishe by Merck Research Lqboratories Division of MERCK & CO., INC. 2001.

6 Mirocha C J, Xie W and Filho, E R: Chemistry and detection of Fusarium mycotoxins. In Leonard K J and Bushnell W R (ed.), Fusarium Head Blight of Wheat and Barley, The American Phytopathological Society, St. Paul, Minn, USA. 2003; p. 144-164.

7 Miller J D: Mycotoxins in small grains and maize: Old problems, new challenges. Food Addit Contam 2008; 25: 219-230.

8 Leslie J F and Summerell B A: The Fusarium Laboratory Manual, Blackwell Publ., Ames, Iowa, USA. 2006; p.388.

9 [Aoki T: Taxonomy of Fusarium genus. Microbiol. Cult. Coll.], 2009; 25: 1-12. Japanese.

10 Bushnell W R, Hazen B E and Pritsch C: Histology and physiology of Fusarium head blight. In Leonard K J and Bushnell W R (ed.), Fusarium Head Blight of Wheat and Barley, The American Phytopathological Society, St. Paul, Minn, USA. 2003; p.44-83.

11 Suga H, Karugia G W, Ward T, Gale, L R, Tomimura K, Nakajima T, Miyasaka A, Koizumi S, Kageyama K and Hyakumachi M: Molecular characterization of the Fusarium graminearum species complex in Japan. Phytopathology 2008; 98: 159-166.

12 Lee J, Chang I Y, Kim H, Yun S H., Leslie J F and Lee Y W: Genetic diversity and fitness of Fusarium gramenearum populations from rice in Korea. Appl Environ Microbiol 2009; 75: 3289-3295.

13 Zhang J B, Li H P., Dang F J, Qu B, Xu Y B, Zhao C S and Liao Y C: Determination of the trichothecene mycotoxin chemotypes and associated geographical distribution and phylogenetic species of the Fusarium graminearum clade from China. Mycol Res 2007; 111: 967-975.

14 Tatsuno T: Scabby wheat intoxication and discovery of nivalenol (a review). Mycotoxins 1997; 45: 11-12.

15 O'Donnell K: Phylogenetic evidence indicates the important mycotoxigenic strains Fn-2, Fn-3, Fn-2B, Fn-M represent a new species of Fusarium. Mycotoxins 1997; 45: 1-10.

16 Kimura M, Tokai T, Takahashi-Ando N, Ohsato S and Fujimura M: Molecular and Genetic Studies of Fusarium Trichothecene Biosynthesis: Pathways, Genes, and Evolution. Bioscience, Biotechnology and Biochemistry 2007; 71(9): 2105-2123.

17 [Suga H: Current studies on Fusarium head blight pathogens]: Journal of General Plant Pathology. 2006; 72(3): 121-134. Japanese.

18 [Yoshizawa T: Human and animal intoxication episodes caused by trichothecene mycotoxins]: Proceedings of the Japanese Association of Mycotoxicolog. 2003; 53: 113-118. Japanese.

19 [Udagawa S: Safety of Rice Grains and Mycotoxins : A Historical Review of Yellow Rice Mycotoxicoses]: The Japanese journal of history of pharmacy. 2004; 39: 321-342. Japanese

20 [Yoshizawa T: A historical review of DON in last 30 years]: Proceedings of the Japanese Association of Mycotoxicolog. 2006; 56: 11-16. Japanese.

21 [Morooka S, Uratuji N, Yoshizawa T, Yamamoto H: Study of the toxic compound in naturally contaminated wheat.] J. Food Hyg. Soc. Jpn. 1972; 13: 368-375.Japanese

22 Yoshizawa T and Morooka N: Deoxynivalenol and its monoacetate: new mycotoxins from Fusarium roseum and moldy barley. Agric Biol Chem 1973; 37: 2933-2934.

23 Vesonder R F, Ciegler A and Jensen A H: Isolation of the emetic principle from Fusarium-infected corn. Appl Environ Mycrobiol 1973; 26: 1008-1010.

24 Miller, J D, Taylor A and Greenhalgh R: Production of deoxynivalenol and related compounds in liquid culture by Fusarium graminearum. Can J Microbiol 1983; 29: 1171-1178.

25 Greenhalgh R, Levandier D, Adams W, Miller J D, Blackwell B A, McAlees A J and Taylor A: Production and characterization of deoxynivalenol and other secondary metabolites of Fusarium culmorum (CMI 14764; HLX 1503). J Agric Food Chem 1986; 34: 98-102.

26 Tatsuno T, Saito M, Enomoto M and Tsunoda H: Nivalenol, a toxic principle of Fusarium nivale. Chem Pharm Bull 1968; 16: 2519-2520.

27 Tatsuno T, Fujimoto Y and Morita Y: Toxicological research on substances from Fusa.rium nivale III. The structure of nivalenol and its monoacetate. Tetrahedron Lett 1969; 33: 2823-2826

28 Ueno Y, Ishikawa Y, Saito-Amakai K and Tsunoda H: Environmental factors influencing the production of fusarenon-X, a cytotoxic mycotoxin of Fusarium nivale Fn2B. Chem Pharm Bull 1970; 18: 304-312.

29 Aoki T and O'Donnell K: Fusarium kyusyuense sp. nov. from Japan. Mycoscience 1998; 39: 1-6

30 [Ueno Y: Future scope of Japanese Association of Mycotoxicology]: Proceedings of the Japanese Association of Mycotoxicolog. 2003; 53: 33-41. Japanese.

31 Opinion of the fusarium toxins part I: deoxynivalenol(DON), Scientific Committee on Food, Health & Consumer Protection Directorate-general, European Commission 1999.

32 Opinion of the scientific committee on food on fusarium toxin PART 4: Nivalenol European Commission 2000.

33 Opinion of the scientific counnrittee on food on fusarium toxins Part 6: Group evaluation of T-2 toxin, HT-2 toxin, nivalenol and deoxynivalenol, Committee on Food, Health & Consumer Protection Directorate-general, European Commission 2002.

34 Yoshizawa T, Hiroaki T and Ohi T: Structure of a novel metabolite from deoxynivalenol, a trichothecene mycotoxin, in animals. Agric Biol Chem 1983; 47(9): 2133-2135.

35 Worrell N R, Mallett A K, Cook W M, Baldwin N C P and Shepherd M J: The role of gut microorganisms in the metabolism of deoxynivalenol administered to rats. Xenobiotica 1989; 19: 25-32.

36 Kollarczik B, Gareis M and Hanelt M: In vitro transformation of the fusarium mycotoxins deoxynivalenol and zearalenone by the normal gut microflora of pigs. Nat Toxins 1994; 2: 105-110

37 He P, Young L G and Forsberg C: Microbial transformation of deoxynivalenol (vomitoxin). Appl Environ Microbiol 1992; 58: 3857-3863.

38 Schatzmayr G, Moll W D, Hofstetter U, Vekiru E, Schatzmayr D and Cheng Y H; Mycotoxin deactivating feed additives in animal nutrition. In Ettle T (ed.), Tagungsband: 5-BOKU-Symposium TIERERNÄHRUNG, Wien, Austria, 2006; p. 47-52.

39 Prelusky D B, Hartin K E, Trenholm H L and Miller J D: Pharmacokinetic fate of carbon-14-labeled deoxynivalenol in swine. Fundam Appl Toxicol 1988; 10: 276-286.

40 Eriksen G S, Pettersson H, Johnsen K and Lindberg J E: Transformation of trichothecenes in ileal digesta and faeces from pigs. Arch. Tierernahr 2002; 56: 263-274.

41 Hedman R and Pettersson H: Transformation of nivalenol by gastrointestinal microbes. Archives of animal nutrition Archiv fur Tierernahrung 1997; 50: 321-329.

42 Seeling K, Danicke S, Valenta H, Van Egmond H P, Schothorst R C, Jekel A A, Lebzien P, Schollenberger M, Razzazi-Fazeli E and Flachowsky G: Effects of Fusarium toxin-contaminated wheat and feed intake level on the biotransformation and carry-over of deoxynivalenol in dairy cows. Food Addit Contam 2006; 23: 1008-1020.

43 Young J C, Zhou T, Yu H, Zhu H and Gong J: Degradation of trichothecene mycotoxins by chicken intestinal microbes. Food Chem Toxicol 2007; 45: 136-143.

44 Eriksen S G and Pettersson H: Lack of de-epoxidation of type B trichothecenes in incubates with human faeces. Food Addit Contam 2003; 20: 579-582.

45 Lake B G, Phillips J C, Walters D G, Bayley D L, Cook M W, Thomas L V, Gilbert J, Startin J R and Baldwin N C P: Studies on the metabolism of deoxynivalenol in the rat. Food Chem Toxicol 1987; 25: 589-592.

46 Danicke S, Valenta H and Doll S: On the toxicokinetics and the metabolism of deoxynivalenol (DON) in the pig. Arch Anim Nutr 2004; 58: 169-180.

47 Goyarts T and Danicke S: Bioavailability of the Fusarium toxin deoxynivalenol (DON) from naturally contaminated wheat for the pig. Toxicol Lett 2006; 163: 171-182.

48 Prelusky D B, Veira D M and Trenholm H L: Plasma pharmacokinetics of the mycotoxin deoxynivalenol following oral and intravenous administration to sheep. J Environ Sci Health B 1985; 20: 603-624.

49 Prelusky D B, Veira D M, Trenholm H L and Hartin K E: Excretion profiles of the mycotoxin deoxynivalenol following oral and intravenous administration to sheep. Fundam Appl Toxicol 1986; 6: 356-363.

50 Prelusky D B, Trenholm H L, Lawrence G A and Scott P M: Nontransmission of deoxynivalenol (vomitoxin) to milk following oral administration to dairy cows. J Environ Sci Health B 1984; 19: 593-609 .

51 Avantaggiato G, Havenaar R and Visconti A: Evaluation of the intestinal absorption of deoxynivalenol and nivalenol by an in vitro gastrointestinal model, and the binding efficacy of activated carbon and other adsorbent materials. Food Chem Toxicol 2004; 42: 817-824.

52 Amuzie C J, Harkema J R and Pestka J J: Tissue distribution and proinflammatory cytokine induction by the trichothecene deoxynivalenol in the mouse: comparison of nasal vs. oral exposure. Toxicology 2008; 248: 39-44.

53 Pestka J J and Amuzie C J: Tissue distribution and proinflammatory cytokine gene expression following acute oral exposure to deoxynivalenol: comparison of weanling and adult mice. Food Chem Toxicol 2008; 46: 2826-2831.

54 Azcona-Olivera J I, Ouyang Y, Murtha J, Chu F S and Pestka J J: Induction of cytokine mRNAs in mice after oral exposure to the trichothecene vomitoxin (deoxynivalenol): Relationship to toxin distribution and protein synthesis inhibition. Toxicol Appl Pharmacol 1995; 133: 109-120.

55 Prelusky D B and Trenholm H L: Tissue distribution of deoxynivalenol in swine dosed intravenously. J Agric Food Chem 1991; 39: 748-751.

56 Prelusky D B, Hamilton R M G, Trenholm H L and Miller J D: Tissue distribution and excretion of radioactivity following administration of carbon-14-labeled deoxynivalenol to white Leghorn hens. Fundam Appl Toxicol 1986; 7: 635-645.

57 Ohta M, Matsumot H, Ishii K and Ueno Y: Metabolism of trichothecene mycotoxins. Part 2: Substrate specificity of microsomal deacetylation of trichothecenes. J Biochem1978; 84: 697-706.

58 Cote L M, Buck W and Jeffrey E: Lack of hepatic microsomal metabolism of deoxynivalenol and its metabolite DOM-1. Food Chem Toxico 1987; 25,4: 291-295.

59 Cote L M, Dahlem A M, Yoshizawa T, Swanson S P and Buck W B: Excretion of deoxynivalenol and its metabolite in milk, urine and feces of lactating dairy cows. J Dairy Sci 1986; 69: 2416-2423.

60 Yoshizawa T, Cote L M, Swanson S P and Buck W B: Confirmation of DOM-1, a deepoxidation metabolite of deoxynivalenol, in biological fluids of lactating cows. Agric Biol Chem 1986; 50: 227-229.

61 Prelusky D B, Veira D M, Trenholm H L and Foster B C: Metabolic fate and elimination in milk, urine and bile of deoxynivalenol following administration to lactating sheep. J Environ Sci Health B 1987; 22: 125-148.

62 Meky F A, Turner P C, Ashcroft A E, Miller J D, Qiao Y L, Roth M J and Wild C P: Development of a urinary biomarker of human exposure to deoxynivalenol. Food Chem Toxicol 2003; 41: 265-273.

63 Prelusky D B, Trenholm H L, Hamilton R M G and Miller J D: Transmission of carbon-14 deoxynivalenol to eggs following oral administration to laying hens. J Agric Food Chem 1987; 35: 182-186.

64 Prelusky D B, Hamilton R M G and Trenholm H L: Transmission of residues to eggs following long-term administration of carbon-14 labelled deoxynivalenol to laying hens. Poultry Sci 1989; 68: 744-748.

65 Charmley E, Trenholm H L, Thompson B K, Vudathala D, Nicholson J W G, Prelusky D B and Charmley L L: Influence of level of deoxynivalenol in the diet of dairy cows on feed intake, milk production, and its composition. J Dairy Sci 1993; 76: 3580-3587.

66 Keese C, Meyer U, Valenta H, Schollenberger M, Starke A, Weber I A, Rehage J, Breves G and Danicke S: No carry over of unmetabolised deoxynivalenol in milk of dairy cows fed high concentrate proportions. Mol Nutr Food Res 2008; 52: 1514-1529.

67 Hunder G, Schumann K, Strugala G, Gropp J, Fichtl B and Forth W: Influence of subchronic exposure to low dietary deoxynivalenol, a trichothecene mycotoxin, on intestinal absorption of nutrients in mice. Food Chem Toxicol 1991; 29: 809-814.

68 Friedman L, Gaines D W, Eppley R, Smith M C, Chi R K and Braunberg R C: Effects of T-2 toxin (T2), deoxynivalenol (DON), zearalenone (Z) and DON+Z on macromolecular synthesis by male rat spleen slices. FASEB J 1996; 10: A458.

69 Awad W A, Rehman H, Bohm J, Razzazi Fazeli E and Zentek J: Effects of luminal deoxynivalenol and L-proline on electrophysiological parameters in the jejunums of laying hens. Poult Sci 2005; 84: 928-932.

70 Awad W A, Razzazi-Fazeli E, Bohm J and Zentek J: Influence of deoxynivalenol on the D-glucose transport across the isolated epithelium of different intestinal segments of laying hens. J Anim Physiol Anim Nutr (Berl) 2007; 91: 175-180.

71 Awad W A, Razzazi-Fazeli E, Bohm J and Zentek J: Effects of B-trichothecenes on luminal glucose transport across the isolated jejunal epithelium of broiler chickens. J Anim Physiol Anim Nutr (Berl) 2008; 92: 225-230 .

72 Szkudelska K, Szkudelski T and Nogowski L: Short-time deoxynivalenol treatment induces metabolic disturbances in the rat. Toxicol Lett 2002; 136: 25-3.1

73 Ehrlich K C and Daigle K W: Protein synthesis inhibition by 8-oxo-12,13-epoxytrichothecenes. Biochem Biophys Acta 1987; 923: 206-213.

74 Betina V: Structure-activity relations among mycotoxins. Chem Biol Interactions 1989; 71: 105-146.

75 Sato N and Ueno Y: Comparative toxicities of trichothecenes. In Rodricks J V, Hesseltine C W and Mehlman M A(ed.): Mycotoxins in Animal and Human Health, Park Forest South, Ilinois, USA. 1977; 295-307.

76 Thompson W L and Wannemacher R W Jr: Structure-function relationships of 12,13-epoxytrichothecene mycotoxins in cell culture: Comparison to whole animal lethality. Toxicon 1986; 24: 985-994.

77 Cetin Y and Bullerman L B: Cytotoxicity of Fusarium mycotoxins to mammalian cell cultures as determined by the MTT bioassay. Food Chem Toxicol 2005; 43:755-764.

78 Tseng H H : Cytotoxicity of food mycotoxins, natural food additives and natural aromas in primary cultured rat hepatocytes. Shi pin Ke xue (Taipei) 1998; 25: 799-812.

79 Isshiki K, Mine H and Shinohara K: Effects of some food additives and mycotoxins on the growth of HuH-6KK cells. Animal Cell Technology 1992; 559-563.

80 Isshiki K, Asano M and Yamashoji S: Cytotoxicity testing for food safety evaluation. In Beuvery E

C, Griffiths J B and Zeijlemaker W P(ed.), Animal Cell Technology: Developments for the 21st Century, Kluwer, Amsterdam, Netherlands, 1995; 999-1003.

81 Wu X, Murphy P, Cunnick J and Hendrich S: Synthesis and characterization of deoxynivalenol glucuronide: its comparative immunotoxicity with deoxynivalenol. Food Chem Toxicol 2007; 45: 1846-1855.

82 Eriksen G S, Pettersson H and Lundh T. Comparative cytotoxicity of deoxynivalenol, nivalenol, their acetylated derivatives and de-epoxy metabolites. Food Chem Toxicol 2004; 42,4: 619-624.

83 Marzocco S, Russo R, Bianco G, Autore G and Severino L: Pro-apoptotic effects of nivalenol and deoxynivalenol trichothecenes in J774A.1 murine macrophages. Toxicol Lett 2009; 189: 21-26.

84 Poapolathep A, Sugita-Konishi Y, Doi K and Kumagai S: The fates of trichothecene mycotoxins, nivalenol and fusarenon-X, in mice. Toxicon 2003; 41: 1047-1054.

85 Hedman R, Pettersson H and Lindberg J E: Absorption and metabolism of nivalenol in pigs. Archives of animal nutrition Archiv fur Tierernahrung 1997; 50: 13-24.

86 Poapolathep A, Poapolathep S, Sugita-Konishi Y, Imsilp K, Tassanawat T, Sinthusing C, Itoh Y and Kumagai S: Fate of fusarenon-X in broilers and ducks. Poult Sci 2008; 87: 1510-1515.

87 Tep J, Videmann B, Mazallon M, Balleydier S, Cavret S and Lecoeur S: Transepithelial transport of fusariotoxin nivalenol: mediation of secretion by ABC transporters. Toxicol Lett 2007; 170: 248-258.

88 Poapolathep A, Sugita-Konishi Y, Phitsanu T, Doi K and Kumagai S: Placental and milk transmission of trichothecene mycotoxins, nivalenol and fusarenon-X, in mice. Toxicon 2004; 44: 111-113.

89 Onji Y, Dohi Y, Aoki Y, Moriyama T, Nagami H, Uno M, Tanaka T and Yamazoe Y: Deepoxynivalenol: a new metabolite of nivalenol found in the excreta of orally administered rats. J Agric Food Chem, 1989; 37: 478-481.

90 Garaleviciene D, Pettersson H and Elwinger K: Effects on health and blood plasma parameters of laying hens by pure nivalenol in the diet. J Anim Physiol Anim Nutr (Berl) 2002; 86: 389-398.

91 Ohtsubo K, Yamad M A and Saito: M. Inhibitory effect of nivalenol, a toxic metabolite of Fusarium nivale on the growth cycle and biopolymer synthesis of HeLa cells. Jpn J med Sci Biol 1968; 21: 185-194.

92 Cundliffe E, Cannon M and Davies J: Mechanism of inhibition of eukaryotic protein synthesis by trichothecene fungal toxins. Proc Natl Acad Sci USA 1974; 71: 30-34.

93 Tanaka T, Matsuda Y, Toyasaki N, Ogawa K, Matsuki Y and Ueno Y: Screening of trichothecene-producing Fusarium species from river sediments by mammalian cell culture techniques. Proc Jap Assoc Mycotoxicol 1978; 5/6: 50-53.

94 Ueno Y, Hosoya M, Morita Y, Ueno I and Tatsuno T: Inhibition of the protein synthesis in rabbit reticulocyte by nivalenol, a toxic principle isolated from Fusarium nivale-growing rice. J Biochem 1968; 64: 479-485.

95 Ueno Y, Nakajima M, Sakai K, Ishii K, Sato N and Shimada N: Comparative toxicology of trichothec mycotoxins: inhibition of protein synthesis in animal cells. J Biochem 1973; 74: 285-296.

96 Gouze M E, Laffitte J, Pinton P, Dedieux G, Galinier A, Thouvenot J P, Loiseau N, Oswald I P and Galtier P: Effect of subacute oral doses of nivalenol on immune and metabolic defence systems in mice. Vet Res 2007; 38: 635-646.

97 [Yosizawa T and Morooka S: Study of the toxic compound in naturally contaminated wheat.-3-] : J. Food Hyg. Soc. Jpn. 1974; 15: 261-269. Japanese.

98 Forsell J H, Jensen R, Tai J H, Witt M, Lin W S and Pestka J J: Comparison of acute toxicities of deoxynivalenol (vomitoxin) and 15-acetyldeoxynivalenol in the B6C3F1 mouse. Food Chem Toxicol 1987; 25: 155-162.

99 Huff W E, Doerr J A, Hamilton P B and Vesonder R F: Acute toxicity of vomitoxin (deoxynivalenol) in broiler chickens. Poultry Sci 1981; 60: 1412-1414.

100 Zielonka L, Wisniewska M, Gajecka M, Obremski K and Gajecki M: Influence of low doses of deoxynivalenol on histopathology of selected organs of pigs. Pol J Vet Sci 2009; 12: 89-95.

101 Forsyth D M, Yoshizawa T, Morooka N and Tuite J: Emetic and refusal activity of deoxynivalenol to swine. Appl Environ Microbiol 1977; 34: 547-552.

102 Pestka J J, Lin W S and Miller E R: Emetic activity of the trichothecene 15-acetyldeoxynivalenol in swine. Food Chem Toxicol 1987; 25: 855-858.

103 Prelusky D B and Trenholm H L; The efficacy of various classes of anti-emetics in preventing deoxynivalenol-induced vomiting in swine. Nat. Toxins 1993; 1: 296-302.

104 Prelusky D B: The effect of low-level deoxynivalenol on neurotransmitter levels measured in pig cerebral spinal fluid. J Environ Sci Health B 1993; 28: 731-761.

105 Prelusky D B; The effect of deoxynivalenol on serotoninergic neurotransmitter levels in pig blood. J Environ Sci Health B 1994; 29: 1203-1218.

106 Foster B C, Trenholm H L, Friend D W, Thompson B K and Hartin K E: Evaluation of different sources of deoxynivalenol (vomitoxin) fed to swine. Can J Anim Sci 1986; 66: 1149-1154.

107 Young L G, McGirr L, Valli V E, Lumsden J H and Lun A: Vomitoxin in corn fed to young pigs. J Anim Sci 1983; 57: 655-664.

108 Friend D W, Trenholm H L, Young J C, Thompson B and Hartin K E: Effects of adding potential vomitoxin (deoxynivalenol) detoxicants or a F. graminearum inoculated corn supplement to wheat diets to pigs. Can J Anim Sci 1984; 64(3): 733-741.

109 Trenholm H L, Hamilton R M, Friend D W, Thompson B K and Hartin K E: Feeding trials with vomitoxin (deoxynivalenol)-contaminated wheat: Effects on swine, poultry, and dairy cattle. J Am Vet Med Assoc 1984; 185: 527-531.

110 Pollmann D S, Koch B A, Seitz L M, Mohr H E and Kennedy G A: Deoxynivalenol-contaminated wheat in swine diets. J Anim Sci 1985; 60: 239-247.

111 Schuh M, Leibetsede, J and Glawischnig E: Chronic effects of different levels of deoxynivaleriol (vomitoxin) on weight gain, feed consumption, blood parameters, pathological as well as histopathological changes in fattening pigs. In Pfannhauser W and Czedik-Eysenberg P B (ed), Proceedings of theInternational IUPAC Symposium on Mycotoxins and Phycotoxins, Vienna, 1982, Vienna, Austrian Chemical Society 5th 1982; 273-276.

112 Hughes D M, Gahl M J, Graham C H and Grieb S L: Overt signs of toxicity to dogs and cats of dietary deoxynivalenol. J Anim Sci 1999; 77: 693-700.

113 Prelusky D B, Yeung J M, Thompson B K and Trenholm H L: Effect of deoxynivalenol on neurotransmitters in discrete regions of swine brain. Arch Environ Contam Toxicol 1992; 22: 36-40

114 Fioramonti J, Dupuy C, Dupuy J and Bueno L: The mycotoxin, deoxynivalenol, delays gastric emptying through serotonin-3 receptors in rodents. J Pharmacol Exp Ther 1993; 266: 1255-1260.

115 Robbana-Barnat S, Loridon-Rosa B, Cohen H, Lafarge-Frayssinet C, Neish G A and Frayssinet C: Protein synthesis inhibition and cardiac lesions associated with deoxynivalenol ingestion in mice. Food Addit Contam 1987; 4: 49-56.

116 Rotter B A, Thompson B K and Rotter R G: Optimization of the mouse bioassay for deoxynivalenol as an alternative to large animal studies. Bull Environ Contam Toxicol 1994; 53: 642-647

117 Rotter B A, Rotter R G, Thompson B K and Trenholm H L: Investigations in the use of mice exposed to mycotoxins as a model for growing pigs. J Toxicol Environ Health 1992; 37: 329-339.

118 Arnold D L, McGuire P F, Nera E A, Karpinski K F, Bickis M G, Zawidzka Z Z, Fernie S and Vesonder R F: The toxicity of orally administered deoxynivalenol (vomitoxin) in rats and mice. Food Chem Toxicol 1986; 24: 935-937, 939-941.

119 Forsell J H, Witt M F, Tai J H, Jensen R and Pestka J J: Effects of 8-week exposure of the B6C3F1 mouse to dietary deoxynivalenol (vomitoxin) and zearalenone. Food Chem Toxicol 1986; 24: 213-219.

120 Arnold D L, Karpinski K F, McGuire P F, Nera E A, Zawidzka Z Z, Lok E, Campbell J S, Tryphonas L and Scott P M: A short-term feeding study with deoxynivalenol (vomitoxin) using rats. Fundam Appl Toxicol 1986; 6: 691-696.

121 Morrissey R E, Norred W P and Vesonder R F: Subchronic toxicity of vomitoxin in Sprague Dawley rats. Food Chem Toxicol 1985; 23: 995-999.

122 Prelusky D B, Gerdes R G, Underhill K L, Rotter B A, Jui, P Y and Trenholm H L: Effects of low-level dietary deoxynivalenol on haematological and clinical parameters of the pig. Nat Toxins 1994; 2: 97-104.

123 Gotz-Schrom S, Schollenberger M, Lauber U, Muller H M and Drochner W: Effect of purified deoxynivalenol in growing pigs-results. Proceedings of the 20th Workshop on Mycotoxins, Bundesanstalt fur Getreide-, Kartoffel- und Fettforschung Detmold. Germany, 1998; 171-175.

124 Lusky K, Gobel R, Tesch D, Tenner G, Haider W, Kruger M and Lippert A: Studies on the effects of ochratoxin A and deoxynivalenol toxicity on the health of pigs and tissue residue concentrations. Tierarztl Umsch 1998; 53: 623-630.

125 Rotter R G, Thompson B K, Trenholm H L, Prelusky D B, Hartin K E and Miller J D: A preliminary examination of potential interactions between deoxynivalenol (DON) and other selected Fusarium metabolites in growing pigs. Can J Anim Sci 1992; 72: 107-11.6

126 Drochner W., Schollenberger M, Gotz S, Lauber U, Tafaj M, and Piepho H. P: Subacute effects of moderate feed loads of isolated Fusarium toxin deoxynivalenol on selected parameters of metabolism in weaned growing piglets. J Anim Physiol Anim Nutr (Berl) 2006; 90,9/10: 421-428.

127 Morris C M, Li Y C, Ledoux D R, Bermudez A J and Rottinghaus G E: The individual and combined effects of feeding moniliformin, supplied by Fusarium fujikuroi culture material, and deoxynivalenol in young turkey poults. Poultry Sci 1999; 78: 1110-1115.

128 Fomenko V N, Adzhigitov F I, Shariya M I and Belova E G: Changes in hemostasis system after administration of mycotoxin deoxynivalenol (vomitoxin) to Macaca-rhesus monkeys. Gematol Transfuziol 1991; 36: 17-19.

129 Iverson F, Armstrong C, Nera E, Truelove J, Fernie S, Scott P, Stapley R, Hayward S and Gunner S: Chronic feeding study of deoxynivalenol in B6C3F1 male and female mice. Terat Carcinog Mutagen 1995; 15: 283-306.

130 Khera K S, Arnold D L, Whalen C, Angers G and Scott P M: Vomitoxin (4-deoxynivalenol): Effects on reproduction of mice and rats. Toxicol Appl Pharmacol 1984; 74: 345-356.

131 Sprando R L, Pestka J, Collins T F X, Rorie J, O'Donnell M, Hinton D and Chirtel S: The effect of vomitoxin (deoxnivalenol) on testicular morphology, testicular spermatid counts and epididymal sperm counts in Il-6ko [B6129-Il6 (Tmlkopf) (Il-6 gene deficient)] and Wt [B6129f2 (wild type to B6129-Il6 with an intact Il-6 gene)] mice. Food Chem Toxicol 1999; 37: 1073-1079

132 Khera K S, Whalen C, Angers G, Vesonder, R F and Kuiper-Goodman T: Embryotoxicity of 4-deoxynivalenol (vomitoxin) in mice. Bull Environ Contam Toxicol 1982; 29: 487-491.

133 Sprando R L, Collins T F, Black T N, Olejnik N, Rorie J I, Eppley R M and Ruggles D I: Characterization of the effect of deoxynivalenol on selected male reproductive endpoints. Food Chem Toxicol 2005; 43: 623-635.

134 Morrissey R E and Vesonder R F: Effect of deoxynivalenol (vomitoxin) on fertility, pregnancy, and postnatal development of Sprague-Dawley rats. Appl Environ Microbiol 1985; 49: 1062-1066

135 Morrissey R E: Teratological study of Fischer rats fed diet containing added vomitoxin. Food Chem Toxicol 1984; 22: 453-457.

136 Tutel'ian V A, Krinitskaia N A, Avreneva L I, Kuzmina E E, Levitskaia A B and Kravchenko L V: Embryotoxic effects of deoxynivalenol mycotoxin (vomitoxin) in rats. Gig Sanit 1991; 10: 49-51.

137 Collins T F, Sprando R L, Black T N, Olejnik N, Eppley R M, Hines F A, Rorie J and Ruggles D I. Effects of deoxynivalenol (DON, vomitoxin) on in utero development in rats. Food Chem Toxicol 2006; 44: 747-757.

138 Khera K S, Whalen C and Angers G: A teratology study on vomitoxin (4-deoxynivalenol) in rabbits. Food Chem Toxicol 1986; 24: 421-424.

139 Wehner F C, Marasas W F O and Thiel P G: Lack of mutagenicity to Salmonella typhimurium of some Fusarium mycotoxins. Appl Environ Microbiol 1978; 35: 659-662.

140 Knasmuller S, Bresgen N, Kassie F, Mersch-Sundermann V, Gelderblom W, Zohrer E and Eckl P. M: Genotoxic effects of three Fusarium mycotoxins, fumonisin B1, moniliformin and vomitoxin in bacteria and in primary cultures of rat hepatocytes. Mutat Res 1997; 391: 39-48.

141 Bradlaw J A, Swentzel K C, Alterman E and Hauswirth J W: Evaluation of purified 4-deoxynivalenol (vomitoxin) for unscheduled DNA synthesis in the primary rat hepatocyte-DNA repair assay. Food Chem Toxicol 1985; 23: 1063-1067.

142 Rogers C G and Heroux-Metcalf C: Cytotoxicity and absence of mutagenic activity of vomitoxin (4-deoxynivalenol) in a hepatocyte-mediated mutation assay with V79 Chinese hamster lung cells. Cancer Lett 1983; 20: 29-35.

143 Hsia C C, Wu, J L, Lu X Q and Li Y S: Natural occurrence and clastogenic effects of nivalenol, deoxynivalenol, 3-acetyl-nivalenol, 15, acetyl-deoxynivalenol, and zearalenone in corn from a high-risk area of oesophageal cancer. Cancer Detect Prev 1988; 13: 79-86.

144 Hsia C C, Wu Z Y, Li Y S, Zhang F and Sun Z T: Nivalenol, a main Fusarium toxin in dietary foods from high-risk areas of cancer of esophagus and gastric cardia in China, induced benign and malignant tumors in mice. Oncol Rep 2004; 12: 449-456.

145 Jone C, Erickson L, Trosko J E and Chang C C: Effect of biological toxins on gap-junctional intercellular communication in Chinese hamster V79 cells. Cell Biol Toxicol 1987; 3: 1-15.

146 Sheu C W, Moreland F M, Lee J K and Dunkel V C: Morphological transformation of BALB/3T3 mouse embryo cells in vitro by vomitoxin. Food Chem Toxicol 1988; 26: 243-245.

147 Sakai A, Suzuki C, Masui Y, Kuramashi A, Takatori K and Tanaka N: The activities of mycotoxins derived from Fusarium and related substances in a short-term transformation assay using v-Ha-ras-transfected BALB/3T3 cells (Bhas 42 cells). Mutat Res 2007; 630(1/2): 103-111.

148 Frankic T, Pajk T, Rezar V, Levart A and Salobir J: The role of dietary nucleotides in reduction of DNA damage induced by T-2 toxin and deoxynivalenol in chicken leukocytes. Food Chem Toxicol 2006; 44: 1838-1844.

149 Tryphonas H, O'Grady L, Arnold D L, McGuire P F, Karpinski K and Vesonder R F: Effect of deoxynivalenol (vomitoxin) on the humoral immunity of mice. Toxicol Lett 1984; 23: 17-24.

150 Tryphonas H, Iverson F, So Y, Nera E A, McGuire P F, O'Grady L, Clayson D B and Scott P M: Effects of deoxynivalenol (vomitoxin) on the humoral and cellular immunity of mice. Toxicol Lett 1986; 30: 137-150.

151 Pestka J J, Tai J H, Witt M F, Dixon D E and Forsell J H: Suppression of immune response in the B6C3F1 mouse after dietary exposure to the Fusarium mycotoxins deoxynivalenol (vomitoxin) and zearalenone. Food Chem Toxicol 1987; 25: 297-304.

152 Robbana-Barnat S, Lafarge-Frayssinet C, Cohen H, Neish G A and Frayssinet C: Immunosuppressive properties of deoxynivalenol. Toxicology 1988; 48: 155-166.

153 Sugita-Konishi Y, Hara K Y, Kasuga F and Kumagai S: The effects of trichothecenes on host defense against infectious diseases. Mycotoxins 1998; 47: 19-23.

154 Sugita-Konishi Y: Effect of trichothecenes on host resistance to bacterial infection. Mycotoxins 2003; 53(2): 141-147.

155 Li M, Harkema J R, Cuff C F and Pestka J J: Deoxynivalenol Exacerbates Viral Bronchopneumonia Induced by Respiratory Reovirus Infection. toxicological sciences 2007; 95(2): 412–426.

156 Landgren C A, Hendrich S and Kohut M L: Low-level dietary deoxynivalenol and acute exercise stress result in immunotoxicity in BALB/c mice. J Immunotoxicol 2006; 3: 173-178.

157 Atroshi F, Rizzo A F, Veijalainen P, Lindberg L A, Honkanen B T, Andersson K, Hirvi T and Saloniemi H: The effect of dietary exposure to DON and T-2 toxin on host resistance and serum

immunoglobulins of normal and mastitic mice. J Anim Physiol Anim Nutr 1994; 71: 223-233.

158 Harvey R B, Kubena L F, Huff W E, Elissalde M H and Phillips T D: Hematologic and immunologic toxicity of deoxynivalenol (DON)-contaminated diets to growing chickens. Bull. Environ. Contam Toxicol 1991; 46: 410-416.

159 Øvernes G, Matre T, Sivertsen T, Larsen H J S, Langseth W, Reitan L J and Jansen J H: Effects of diets with graded levels of naturally deoxynivalenol-contaminated oats on immune response in growing pigs. J Vet Med A Physiol Pathol Clin Med 1997; 44: 539-550.

160 Ferrari L, Cantoni A M, Borghetti P, De Angelis E and Corradi A: Cellular immune response and immunotoxicity induced by DON (deoxynivalenol) in piglets. Vet Res Commun 2009; 33 Suppl 1: 133-135.

161 Accensi, F, Pinton P, Callu P, Abella-Bourges N, Guelfi J F, Grosjean F and Oswald I P: Ingestion of low doses of deoxynivalenol does not affect hematological, biochemical, or immune responses of piglets. J Anim Sci 2006; 84: 1935-1942.

162 Pestka J J, Moorman M A and Warner R L: Dysregulation of IgA production and IgA nephropathy induced by the trichothecene vomitoxin. Food Chem Toxicol 1989; 27: 361-368.

163 Greene D M, Azcona-Olivera J I and Pestka J J: Vomitoxin (deoxynivalenol) induced IgA nephropathy in the B6C3F1 mouse: Dose-response and male predilection. Toxicology 1994; 92: 245-260.

164 Pestka J J, Dong W, Warner R L, Rasooly L and Bondy G S: Effect of dietary administration of the trichothecene vomitoxin (deoxynivalenol) on IgA and IgG secretion by Peyer's patch and splenic lymphocytes. Food Chem Toxicol 1990; 28: 693-699.

165 Pestka J J, Dong W, Warner R L, Rasooly L, Bondy G S and Brooks K H: Elevated membrane IgA positive and CD4 positive (T helper) populations in murine Peyer's patch and splenic lymphocytes during dietary administration of the trichothecene vomitoxin (deoxynivalenol). Food Chem Toxicol 1990; 28: 409-415, 417-420.

166 Bondy G S and Pestka J J: Dietary exposure to the trichothecene vomitoxin (deoxynivalenol) stimulates terminal differentiation of Peyer's patch B cells to IgA secreting plasma cells. Toxicol Appl Pharmacol 1991; 108: 520-530.

167 Yan D, Zhou H R, Brooks K H and Pestka J J: Potential role for IL-5 and IL-6 in enhanced IgA secretion by Peyer's patch cells isolated from mice acutely exposed to vomitoxin. Toxicology 1997; 122: 145-158.

168 Gouze M E, Laffitte J, Dedieu G, Galinier A: Thouvenot J P, Oswald I P and Galtier P: Individual and combined effects of low oral doses of deoxynivalenol and nivalenol in mice. Cell Mol Biol (Noisy-le-grand) 2005; Suppl.51: 809-817.

169 Kim E J, Jeong S H, Cho J H, Ku H O, Pyo H M, Kang H G and Choi K H: Plasma haptoglobin and immunoglobulins as diagnostic indicators of deoxynivalenol intoxication. J Vet Sci 2008; 9 257-266

170 Dong W M and Pestka J J: Persistent dysregulation of IgA production and IgA nephropathy in the B6C3F1 mouse following withdrawal of dietary vomitoxin (deoxynivalenol). Fundam Appl Toxicol 1993; 20, 38-47.

171 Banotai C, Greene-Mcdowelle D M; Azcona-Olivera J I and Pestka J J: Effects of intermittent vomitoxin exposure on body weight, immunoglobulin levels and haematuria in the B6c3f(1) mouse. Food Chem Toxicol 1999; 37: 343-350.

172 Pestka J J and Zhou H R: Interleukin-6-deficient mice refractory to IgA dysregulation but not anorexia induction by vomitoxin (deoxynivalenol) ingestion. Food Chem Toxicol 2000; 38(7): 565-75.

173 Jia Q and Pestka J J: Role of cyclooxygenase-2 in deoxynivalenol-induced immunoglobulin a nephropathy. Food Chem Toxicol 2005; 43: 721-728.

174 Banotai C, Azcona-Olivera J I, Greene-McDowelle D M and Pestka J J: Effects of vomitoxin ingestion on murine models for systemic lupus erythematosus. Food Chem Toxicol 1999; 37: 533-543.

175 Pinton P, Accensi F, Beauchamp E, Cossalter A M, Callu P, Grosjean F, and Oswald I P: Ingestion of deoxynivalenol (DON) contaminated feed alters the pig vaccinal immune responses. Toxicol Lett 2008; 177: 215-222.

176 Drochner W, Schollenberger M, Piepho H P, Götz S, Lauber U, Tafaj M, Klobasa F, Weiler U, Claus R and Steffl M: Serum IgA-promoting effects induced by feed loads containing isolated deoxynivalenol (DON) in growing piglets. J Toxicol Environ Health 2004; A 67: 1051-1067.

177 Bergsjo B, Langseth W, Nafstad I, Jansen J H and Larsen H J: The effects of naturally deoxynivalenol-contaminated oats on the clinical condition, blood parameters, performance and carcass composition of growing pigs. Vet Res Commun 1993; 17: 283-294.

178 Kinser S, Jia Q, Li M, Laughter A, Cornwell P, Corton J C and Pestka J: Gene expression profiling in spleens of deoxynivalenol-exposed mice: immediate early genes as primary targets. J Toxicol Environ Health A 2004; 67: 1423-1441.

179 Ouyang Y L, Azcona Olivera J I, Murtha J and Pestka J J; Vomitoxin-mediated IL-2, IL-4, and IL-5 superinduction in murine CD4+ T cells stimulated with phorbol ester calcium ionophore: Relation to kinetics of proliferation. Toxicol Appl Pharmacol 1996; 138: 324-334.

180 Li S G, Ouyang Y L, Yang G H and Pestka J J: Modulation of transcription factor Ap-1 activity in murine El-4 thymoma cells by vomitoxin (Deoxynivalenol). Toxicol Appl Pharmacol 2000; 163: 17-25.

181 Li S G, Ouyang Y L, Dong W M, Pestka J J and Dong W M: Superinduction of IL-2 gene expression by vomitoxin (deoxynivalenol) involves increased mRNA stability. Toxicol Appl Pharmacol 1997; 147, 331-342.

182 Gray J S and Pestka J J: Transcriptional regulation of deoxynivalenol-induced IL-8 expression in human monocytes. Toxicol Sci 2007; 99: 502-511.

183 Zhou H R, Yan D and Pestka J J: Differential cytokine mRNA expression in mice after oral exposure to the trichothecene vomitoxin (deoxynivalenol): dose response and time course. Toxicol Appl Pharmacol 1997; 144: 294-305.

184 Zhou H R, Yan D and Pestka J J: Induction of cytokine gene expression in mice after repeated and subchronic oral exposure to vomitoxin (Deoxynivalenol): differential toxin-induced hyporesponsiveness and recovery. Toxicol Appl Pharmacol 1998; 151: 347-358.

185 Moon Y and Pestka J J: Cyclooxygenase-2 mediates interleukin-6 upregulation by vomitoxin (deoxynivalenol) in vitro and in vivo. Toxicol Appl Pharmacol 2003; 187: 80-88.

186 Kinser S, Li M, Jia Q and Pestka J J: Truncated deoxynivalenol-induced splenic immediate early gene response in mice consuming (n-3) polyunsaturated fatty acids. J Nutr Biochem 2005; 16: 88-95.

187 Amuzie C J, Shinozuka J and Pestka J J: Induction of suppressors of cytokine signaling by the trichothecene deoxynivalenol in the mouse. Toxicol Sci 2009; 111(2):277-87.

188 Amuzie C J and Pestka J J: Suppression of insulin-like growth factor acid-labile subunit expression a novel mechanism for deoxynivalenol -induced growth retardation. Toxicol Sci 2010; 113(2):412-21.

189 Pestka J J, Yan D and King L E: Flow cytometric analysis of the effects of in vitro exposure to vomitoxin (deoxynivalenol) on apoptosis in murine T, B and IgA+ cells. Food Chem Toxicol 1994; 32: 1125-1129, 1131-1136.

190 Rizzo A F, Atroshi F, Hirvi T and Saloniemi H: The hemolytic activity of deoxynivalenol and T-2 toxin. Nat Toxins 1992; 1: 106-110.

191 Johannisson A, Bjorkhag B, Hansson W, Gadhasson I L and Thuvander A: Effects of four trichothecene mycotoxins on activation marker expression and cell proliferation of human lymphocytes in culture. Cell Biol Toxicol 1999; 15: 203-215.

192 Parent-Massin D and Thouvenot D: In vitro toxicity of trichothecenes on rat haematopoietic progenitors. Food Addit Contam 1995; 12: 41-49.

193 Lautraite S, Parent-Massin D, Rio B and Hoellinger H: In vitro toxicity induced by deoxynivalenol (DON) on human and rat granulomonocytic progenitors. Cell Biol Toxicol 1997; 13: 175-183.

194 Parent-Massin D, Fuselier R and Thouvenot D: In vitro toxicity of trichothecenes on human haematopoietic progenitors. Food Addit Contam 1994; 11: 441-447.

195 Rio B, Lautraite S and Parent-Massin D: In vitro toxicity of trichoethecenes on human erythroblastic progenitors. Human Exp Toxicol 1997; 16: 673-679.

196 Kasuga F, Hara-K Y, Saito N, Kumagai S and Sugita-Konishi Y: In vitro effect of deoxynivalenol on the differentiation of human colonic cell lines Caco-2 and T84. Mycopathologia 1998; 142: 161-167.

197 Pinton P, Nougayrede J P, Del Rio J C, Moreno C, Marin D E, Ferrier L, Bracarense A P, Kolf-Clauw M and Oswald I P: The food contaminant deoxynivalenol, decreases intestinal barrier permeability and reduces claudin expression. Toxicol Appl Pharmacol 2009; 237: 41-48.

198 Kolf-Clauw M, Castellote J, Joly B, Bourges-Abella N, Raymond-Letron I, Pinton P and Oswald I P: Development of a pig jejunal explant culture for studying the gastrointestinal toxicity of the mycotoxin deoxynivalenol: histopathological analysis. Toxicol In Vitro 2009; 23(8): 1580-4.

199 Sugiyama K I, Muroi M and Tanamoto K I: Deoxynivalenol and nivalenol inhibit lipopolysaccharide-induced nitric oxide production by mouse macrophage cells. Toxicol Lett 2010; 192(2):150-4.

200 Shi Y and Pestka J J: Mechanisms for suppression of interleukin-6 expression in peritoneal macrophages from docosahexaenoic acid-fed mice. J Nutr Biochem 2009; 20: 358-68.

201 Zhou H R, Lau A S and Pestka J J: Role of double-stranded RNA-activated protein kinase R (PKR) in deoxynivalenol-induced ribotoxic stress response. Toxicol Sci 2003; 74: 335-344.

202 Ryu J-C, Ohtsubo K, Izumiyama N, Nakamura K, Tanaka T, Yamamura H and Ueno Y: The acute and chronic toxicities of nivalenol in mice. Fundam Appl Toxicol 1988; 11: 38-47.

203 [Kawasaki Y, Uchida Y, Sekiguchi K, Matsumoto K, Ochiai T, Usui A, Nakamichi S, Furuya T and Kurokawa Y: Single and repeated oral toxicity study of Nivalenol in F344 rats]: Food Hyg. Soc. Jpn. 1990; 31: 144-154. Japanese.

204 Ueno Y: Developments in Food Science. IV Trichothecenes. In Ueno Y(ed.), Chemical, Biological and Toxicological Aspects, Amsterdam, Elsevier, General toxicology 1983; p. 135-146.

205 Ueno Y, Ueno I, Iitoi Y, Tsunoda H, Enomoto M and Ohtsubo K: Toxicological approaches to the metabolites of Fusaria. III. Acute toxicity of fusarenon-X. Jpn Jpn J exp Med 1971; 41,6: 521-539.

206 Ryu JC, Ohtsubo K, Izumiyarna N, Mori M, Tanaka T and Ueno Y: Effects of nivalenol on the bone marrow in mice. J Toxicol Sci 1987; 12: 11-2.1

207 Yamamura H, Kobayashi T, Ryu L C and Ueno Y: Subchronic feeding studies with nivalenol in C57BL/6 mice. Food Chem Toxicol 1989; 27: 585-590.

208 Yabe T, Hashimota H, Sekijima M, Ddegawa M, Hashimoto Y, Tashiro F and Ueno Y: Effects of nivalenol on hepatic drug-metabolizing activirty in rats. Food Chem Toxicol 1993; 31: 573-577, 579-581.

209 Kubosaki A, Aihara M, Park B J, Sugiura Y, Shibutani M, Hirose M, Suzuki Y, Takatori K and Sugita-Konishi Y: Immunotoxicity of nivalenol after subchronic dietary exposure to rats. Food Chem Toxicol 2008; 46: 253-258.

210 Takahashi M, Shibutani M, Sugita-Konishi Y, Aihara M, Inoue K, Woo G H, Fujimoto H and Hirose M: A 90-day subchronic toxicity study of nivalenol, a trichothecene mycotoxin, in F344 rats. Food Chem Toxicol 2008; 46: 125-135.

211 Hedman R, Thuvander A, Gadhasson I, Reverter M and Pettersson H: Influence of dietary nivalenol exposure on gross pathology and selected immunological parameters in young pigs. Nat Toxins 1997; 5: 238-246.

212 Hedman R, Pettersson H, Engstrom B, Elwinger K and Fossum O: Effects of feeding nivalenol-contaminated diets to male broiler chickens. Poult Sci 1995; 74: 620-625.

213 Ohtsubo K, Ryu J C, Nakamura K, Izumiyama N, Tanaka T, Yamamura H, Kobayashi T and Ueno Y: Chronic toxicity of nivalenol in female mice: a 2-year feeding study with Fusarium nivale Fn 2b-moulded rice. Food Chem Toxicol 1989; 27: 591-598.

214 Ueno Y, Kobayashi T, Yamamura H, Kato T, Tashiro F, Nakamura K and Ohtsubo K: Effect of long-term feeding of nivalenol on aflatoxin-B1-initiated hepatocarcinogenesis in mice. In O'Neill I K, Chen J and Bartsch H(ed). Relevance to Human Cancer of N-Nitroso Compounds, Tobacco Smoke and Mycotoxins (IARC Scientific Publications No. 105), Lyon, IARC 1991; 105: 420-423.

215 Ueno Y, Yabe T, Hashimoto H, Sekijima M, Masuda T, Kim D J, Hasegawa R and Ito N: Enhancement of GST-P-positive liver cell foci development by nivalenol, a trichothecene mycotoxin. Carcinogenesis 1992; 13: 787-791.

216 Saito M, Enomoto M and Tatsuno T: Radiomimetic biological properties of the new scirpene metabolites of Fusarium nivale. Gann 1969; 60: 599-603.

217 [Ito Y, Ueno Y, Tanaka T, Nakamura K and Ootsubo K: Reproductive and Developmental toxicity of Nivalenol in rats.] Proceedings of the Japanese Association of Mycotoxicolog. 1988; 27: 33-36. Japanese.

218 Thust R, Kneist S and Huhne V: Genotoxicity of Fusarium mycotoxins (nivalenol, fusarenon-X, T-2 toxin, and zearalenone) in Chinese hamster V79-E cells in vitro. Arch Geschwulstforsch 1983; 53: 9-15.

219 Tsuda S, Kosaka Y, Murakami M, Matsuo H, Matsusaka N, Taniguchi K and Sasaki Y F: Detection of nivalenol genotoxicity in cultured cells and multiple mouse organs by the alkaline single-cell gel electrophoresis assay. Mut Res 1998; 415: 191-200.

220 [Hayashi M: The development of the genotoxicity assays using transgenic mice. A part of the studies for the development and the application of the new methods to evaluate the carcinogenicity using experimental animals (Project manager; Yuji Kurokawa): Annual report of a Grant-in-Aid for cancer research in H13(FY2001)]; 557-563: the Ministry of Health, Labor and Welfare of Japan. Japanese

221 Hinoshita F, Suzuki Y, Yokoyama K, Hara S, Yamada A, Ogura Y, Hashimoto H, Tomura S, Marumo F and Ueno Y: Experimental IgA nephropathy induced by a low-dose environmental mycotoxin, nivalenol. Nephron 1997; 75: 469-478.

222 Poapolathep A, Nagata T, Suzuki H, Kumagai S and Doi K: Development of early apopotosis and changes in lymphocyte subsets in lymphoid organs of mice orally inoculated with nivalenol. Exp Mol Pathol 2003; 75: 74-79.

223 Choi C Y, Nakajima A H, Kaminogowa S and Sugita-Konishi Y: Nivalenol inhibits total and antigen-specific IgE production in mice. Toxicol Appl Pharmacol 2000; 165: 94-98.

224 [Hinosita H, Ogura Y, Hara S, Yamada A, Marusige A, Ueno Y, Hiroi T and Kiyono H: Involvement of the gut-associated lymphoid tissue (GALT) in the Nivalenol-induced IgA nephropathy in mice]: Digestive organ and immunology 1996; 33: 30-33. Japanese.

225 Luongo D, Severino L, Bergamo P, D'Arienzo R and Rossi M: Trichothecenes NIV and DON modulate the maturation of murine dendritic cells. Toxicon 2010; 55(1): 73-80

226 Poapolathep A, Ohtsuka R, Kiatipattanasakul W, Ishigami N, Nakayama H and Doi K: Nivalenol--induced apoptosis in thymus, spleen and Peyer's patches of mice. Exp Toxicol Pathol 2002; 53: 441-446.

227 Forsell J H and Pestka J: Relation of 8-ketotrichothecene and zearalenone analog structure to inhibition of mitogen-induced human lymphocyte blastogenesis. Appl Environ Microbiol 1985; 50: 1304-1307.

228 Thuvander A, Wikman C and Gadhasson I: In vitro exposure of human lymphocytes to trichothecenes: individual variation in sensitivity and effects of combined exposure on lymphocyte function. Food Chem Toxicol 1999; 37: 639-648.

229 Luongo D, De Luna R, Russo R and Severino L: Effects of four Fusarium toxins (fumonisin B(1), alpha-zearalenol, nivalenol and deoxynivalenol) on porcine whole-blood cellular proliferation.

Toxicon 2008; 52: 156-162.

230 Madhyastha M S, Marquardt R R and Abramson D: Structure-activity relationships and interactions among trichothecene mycotoxinsas assessed by yeast bioassay. Toxicon 1994; 32: 1147-1152

231 443:Creppy E E: Update of survey, regulation and toxic effects of mycotoxins in Europe. Toxicol Lett 2002; 127: 19-28.

232 Luo X: Food poisoning caused by Fusarium toxins. In Proceedings of the Second Asian Conference on Food Safety, ILSI, Thailand, 1994; p.129–136.

233 Luo Y, Yoshizawa T and Katayama T: Comparative study on the natural occurrence of Fusarium mycotoxins (trichothecenes and zearalenone) in corn and wheat from high- and low-risk areas for human esophageal cancer in China. Appl Environ Microbiol 1990; 56: 3723-3726.

234 Gao H P and Yoshizawa T: Further study on fusarium mycotoxins in corn and wheat from a high-risk area for human esophageal cancer in China. Mycotoxins 1997; 45: 51-55.

235 Wang D S, Liang Y X, Iijima K, Sugiura Y, Tanaka T, Chen, G., Yu, S Z and Ueno Y: Co-contamination of mycotoxins in corn harvested in Haimen, a high risk area of primary liver cancer in China. Mycotoxins 1995; 41: 67-70.

236 Luo Y, Yoshizawa T, Yang J S, Zhang S Y and Zhang B J: A survey of Fusarium mycotoxins (trichothecenes, zearalenone and fusarochromanone) in corn and wheat samples from Shaanxi and Shanxi Provinces, China. Mycotoxin Res 1992; 8: 85-91.

237 Bhat R V, Beedu S R, Ramakrishna Y and Munshi K L: Outbreak of trichothecene mycotoxicosis associated with consumption of mould-damaged wheat production in Kashmir Valley, India. Lancet 1989; 1: 35-37.

238 Bhat R V, Ramakrishna Y and Sashidhar R B: Outbreak of mycotoxicosis in Kashmir Valley, India. Nutr News Natl Inst Nutr 1989; 10: 5.

239 Summary report of the seventy-second meeting of JECFA, 2010.
(http://www.who.int/foodsafety/chem/summary72_rev.pdf)

240 Atkinson H A and Miller K: Inhibitory effect of deoxynivalenol, 3-acetyldeoxynivalenol and zearalenone on induction of rat and human lymphocyte proliferation. Toxicol Lett 1984; 23(2): 215-221.

241 Eriksen G S, Pettersson H and Lindberg J E: Absorption, metabolism and excretion of 3-acetyl DON in pigs. Arch Tierernahr 2003; 57: 335-345.

242 [Yosizawa T: A survey of deoxinivalenol and nivalenol contamination in rice grains in Japan. A part of the urgent studies for the establishment of standards for deoxinivalenol in wheat etc. Annual report of a Grant-in-Aid for the Special research (Project manager; Susumu Kumagai) in H14 (FY2003)]; 49-63: the Ministry of Health, Labor and Welfare of Japan. Japanese.

243 Discussion Paper On Deoxynivalenol, Joint Fao/Who Food Standards Programme Codex Committee On Contaminants In Foods, First Session, Beijing, China, Codex 2007

244 Collection of Occurrence Data of Fusarium Toxins in Food and Assessment of Dietary Intake by the Population of EU Member States 2003 SCOOP Task 3.2.10 Final Report
(http://europa.eu.int/comm/food/fs/scoop/task3210.pdf.)

245 [Monitoring results of pesticide residues in rice and wheat: Ministry of Agriculture, Forestry and Fisheries of Japan]. Japanese.
(http://www.maff.go.jp/j/syouan/nouan/kome/k_beibaku/index.html)

246 [Monitoring results of mycotoxins in cereals: Ministry of Agriculture, Forestry and Fisheries of Japan]. Japanese.
(http://www.maff.go.jp/j/syouan/seisaku/risk_analysis/priority/kabidoku/tyosa/index.html)

247 [Wheat supply and demand outlook in H19 (FY2007): Ministry of Agriculture, Forestry and Fisheries of Japan]. Japanese.

248 [Wheat supply and demand outlook in H21 (FY2009): Ministry of Acgriculture, Forestry and Fisheries]. Japanese.

249 [Food safety risk profile; Deoxynivalenol (Discussion paper): 2009 Ministry of Acgriculture, Forestry and Fisheries of Japan]. Japanese.
(http://www.maff.go.jp/j/syouan/seisaku/risk_analysis/priority/kabidoku/pdf/chem_don.pdf)

250 [Food safety risk profile; Nivalenol (Discussion paper): 2009 Ministry of Agriculture, Forestry and Fisheries of Japan]. Japanese.
(http://www.maff.go.jp/j/syouan/seisaku/risk_analysis/priority/kabidoku/pdf/chem_niv.pdf)

251 [Kumagai S: Survey of the occurrences of afoatoxin M1 in milk and deoxinivalenol in cereals in Japan. A part of the studies for the risk assessment of mycotoxins in foods. Annual report of a Grant-in-Aid for the Special research (Project manager; Susumu Kumagai) in H13 (FY2002)]; 1-10: the Ministry of Health, Labor and Welfaer of Japan. Japanese.

252 [Konishi Y: Occurrence of mycotoxins in foods: Report for MHLW in H15 (FY2004)]: the Ministry of Health, Labor and Welfare of Japan. Japanese.

253 [Sato T: Estimation of the daily exposure to deoxynivalenol from wheat consumption in Japan using Monte-Carlo simulation. A part of the studies for the toxicity and exposure assessments of mycotoxins in foods. Annual report of a Grant-in-Aid for the food safty (Project manager; Yoko Konishi) of H17 (FY2006)] ;191-193: the Ministry of Health, Labor and Welfare of Japan. Japanese.

254 [Daily intake of contaminants from food: Report for MHLW of H17 (FY2006).]: the Ministry of Health, Labor and Welfare of Japan. Japanese.

255 [Ichinoe M: Relation of weather conditions to incidence of wheat scab and Fusarium-toxins]: Proceedings of the Japanese Association of Mycotoxicolog. 2003; 53:5-10. Japanese.

256 [Sato T: Estimation of the daily exposure to nivalenol from wheat consumption in Japan using Monte-Carlo simulation. A part of the studies for the toxicity and exposure assessments of mycotoxins in foods. Annual report of a Grant-in-Aid for the food safty (Project manager; Yoko Konishi) in H17 (FY2006)] ;191-193: the Ministry of Health, Labor and Welfare of Japan. Japanese.

257 [Kumagai, S: Survey of DON and NIV contamination in wheat grain and flower. A part of a Grant-in-Aid for the urgent studies for the establishment of standards for deoxinivalenol in wheat etc. Annual report of a special research (conducted by Susumu Kumagai) in H14 (FY2003)], the Ministry of Health, Labor and Welfare of Japan. Japanese.

258 Sugita-Konishi Y, Park B J, Kobayashi-Hattori K, Tanaka T, Chonan T, Yoshikawa K and Kumagai S: Effect of cooking process on the deoxynivalenol content and its subsequent cytotoxicity in wheat products. Biosci Biotechnol Biochem 2006; 70(7):1764-1768.

259 Sugiyama K, Tanaka H, Kamata Y, Tanaka T, Sugita-Konishi Y: A reduced rate of deoxynivalenol and nivalenol during bread production from wheat flour in Japan. Mycotoxins 2009; 59(1): 1-6.

260 Yumbe-Guevara B E, Imoto T and Yoshizawa T: Effects of heating procedures on deoxynivalenol, nivalenol and zearalenone levels in naturally contaminated barley and wheat. Food Addit Contam 2003; 20(12):1132-1140.

261 Visconti A, Haidukowski EM, Pascale M, Silvestri M. Reduction of deoxynivalenol during durum wheat processing and spaghetti cooking. Toxicol Lett. 2004; 10;153(1):181-189.

262 Scott P M, Kanhere S R, Dexter J E, Brennan P W and Trenholm H L: Distribution of the trichothecene mycotoxin deoxynivalenol (vomitoxin) during the milling of naturally contaminated hard red spring wheat and its fate in baked products. Food Addit Contam 1984; 1(4): 313-323.

263 Boyacioglu D, Hettiarachchy N S and D'Appolonia B L: Additives affect deoxynivalenol (vomitoxin) flour during breadbaking. Journal of Food Science 1993; 58 (2): 416-418.

264 Scott P M, Kanhere S R, Lau P Y: Dexter J E and Greenhalgh R: Effects of experimental flour milling and breadmaking on retention of deoxynivalenol (vomitoxin) in hard red spring wheat. Cereal Chem 1983; 60: 421-424.

265 El-Banna A A, Lau P Y and Scott P M: Fate of mycotoxins during processing offoodstuffs. II. Deoxynivalenol. (vomitoxin) during making of Egyptian bread. J Food Prot 1983; 46: 484-486

266 Seitz L M and Bechtel D B: Chemical, physical, and microscopal studies of scab-infected hard red

winter wheat. J Agric Food Chem 1985; 33: 373-377.

267 Young J C, Fulcher R G, Hayhoe J H, Scott P M and Dexter J E: Effect of milling and baking on deoxynivalenol (vomitoxin) content of eastern Canadian wheats. J Agric Food Chem 1984; 32: 659-664.

268 Schwarz P B, Casper H H and Beattie S. Fate and development of naturally occurring Fusarium mycotoxins during malting and brewing. J Am Soc Brew Chem 1995; 53: 121–127.

269 Lancova K, Hajslova J, Poustka J, Krplova A, Zachariasova M, Dostalek P and Sachambula L: Transfer of Fusarium mycotoxins and 'masked' deoxynivalenol (deoxynivalenol-3-glucoside) from field barley through malt to beer. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 2008; 25(6): 732-744.

270 Kushiro M: Effects of Milling and Cooking Processes on the Deoxynivalenol Content in Wheat. Int J Mol Sci 2008; 9: 2127-2145.

271 Basilico M Z, Sartore A L and Basilico J C: Detoxification of corn contaminated with deoxynivalenol (vomitoxin) using sodium bisulfite. Effect on Wistar rats. Inf Technol 1997; 8: 57-63.

272 Harvey R B, Edrington T S, Kubena L F, Elissalde M H, Casper H H, Rottinghaus G E and Turk J R: Effects of dietary fumonisin B1-containing culture material, deoxynivalenol-contaminated wheat, or their combination on growing barrows. Am J Vet Res 1996; 57: 1790-1794.

273 Rotter, B.A., Thompson, B.K., Lessard, M., Trenholm, H.L. and Tryphonas H: Influence of low-level exposure to fusarium mycotoxins on selected immunological and hematological parameters in young swine. Fundam Appl Toxicol 1994; 23: 117-124.

274 Friend D W, Thompson B K, Trenholm H L, Boermans H J, Hartin K E and Panich P L: Toxicity of T-2 toxin and its interaction with deoxynivalenol when fed to young pigs. Can J Anim Sci 1992; 72: 703-711.

275 Rotter B A: Thompson B K and Lessard M: Effects of deoxynivalenol-contaminated diet on performance and blood parameters in growing swine. Can J Anim Sci 1995; 75: 297-302.

276 Bergsjo B, Matre T and Nafstad I: Effects of diets with graded levels of deoxynivalenol on performance in growing pigs. Zentralbl Veterinarmed A 1992; 39: 752-758.

277 Bimczok D, Doll S, Rau H, Goyarts T, Wundrack N, Naumann M, Danicke S and Rothkotter H J: The Fusarium toxin deoxynivalenol disrupts phenotype and function of monocyte-derived dendritic cells *in vivo* and *in vitro*. Immunobiology 2007; 212,8: 655-666.

278 Johnson P J, Casteel S W and Messer N T: Effect of feeding deoxynivalenol (vomitoxin)-contaminated barley to horses. J Vet Diagn Invest 1997; 9: 219-221.

279 Ingalls J R: Influence of deoxynivalenol on feed consumption by dairy cows. Anim Feed Sci Technol 1996; 60: 297-300.

280 Anderson V L, Boland E W and Casper H H: Effects of vomitoxin (DON) from scab infested barley on performance of feedlot and breeding beef cattle. J Anim Sci 1996; 74: 208.

281 Harvey R B, Kubena L F, Corrier D E, Witzel D A, Phillips T D and Heidelbaugh N D: Effects of deoxynivalenol in a wheat ration fed to growing lambs. Am J Vet Res 1986; 47: 1630-1632.

282 Harvey R B, Kubena L F, Rottinghaus G E, Turk J R, Casper H H and Buckley S A: Moniliformin from Fusarium fujikuroi culture material and deoxynivalenol from naturally contaminated wheat incorporated into diets of broiler chicks. Avian Dis 1997; 41: 957-963.

283 Kubena L F, Huff W E, Harvey R B, Phillips T D and Rottinghaus G E: Individual and combined toxicity of deoxynivalenol and T-2 toxin in broiler chicks. Poultry Sci 1989; 68: 622-626.

284 Kubena L F, Edrington T S, Harvey R B, Buckley S A, Phillips T D, Rottinghaus G E and Casper H H: Individual and combined effects of fumonisin B1 present in Fusarium moniliforme culture material and T-2 toxin or deoxynivalenol in broiler chicks. Poultry Sci 1997; 76: 1239-1247.

285 Bergsjo B and Kaldhusdal M: No association found between the ascites syndrome in broilers and feeding of oats contaminated with deoxynivalenol up to thirty-five days of age. Poultry Sci 1994;

73: 1758-1762.

286 Leitgeb R, Lew H, Wetscherek W, Bohm J and Quinz A: Influence of fusariotoxins on growing and slaughtering perfomance of broilers. Bodenkultur 1999; 50: 57-66.

287 Boston S, Wobeser G and Gillespie M: Consumption of deoxynivalenol-contaminated wheat by mallard ducks under experimental conditions. J Wildl Dis 1996; 32: 17-22.