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Toxicology and occurrence of nivalenol, fusarenon X, diacetoxyscirpenol, neosolaniol and 3- and 15-acetyldeoxynivalenol: a review of six trichothecenes

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Abstract

To assess the potential health effects of the trichothecenes nivalenol (NIV), fusarenon X (FusX), diacetoxyscirpenol (DAS), neosolaniol (NeoSol) and 3- and 15-acetyldeoxynivalenol (3-Ac-DON, 15-Ac-DON), toxicology data available on these compounds were reviewed to derive, where possible, tolerable daily intakes (TDIs). Also, their occurrence in cereal grains, animal feed and human foodstuffs was investigated. The six trichothecenes are mycotoxins produced by *Fusarium* spp. NIV was the one most often found in food commodities. FusX, DAS, NeoSol and 3- and 15-Ac-DON have rarely been reported to occur, most likely because they were not routinely monitored for. It was therefore recommended to pay more attention to FusX, DAS, NeoSol and 3- and 15-Ac-DON in routine monitoring programs. The toxicology data available on FusX, DAS, 3-Ac-DON, 15-Ac-DON and NeoSol were too limited to derive TDIs. Only for NIV sufficient toxicology data were available to derive a temporary TDI of 0.7 µg/kg bw.

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Samenvatting

Mycotoxinen zijn door schimmels geproduceerde, van nature voorkomende toxische stoffen. Indien deze mycotoxinen terecht komen in diervoeders of in voor mensen bestemd voedsel, dan kunnen ze toxische verschijnselen induceren en aldus een bedreiging vormen voor de gezondheid van dier en mens. Trichothecenen vormen een belangrijke groep mycotoxinen, en alhoewel er meer dan 150 verschillende trichothecenen bekend zijn komt slechts een klein aantal daarvan daadwerkelijk voor in granen, diervoeders en voedingsmiddelen. Dit zijn deoxynivalenol (DON), nivalenol (NIV), diacetoxyscirpenol (DAS), T-2 toxine (T-2) en, in mindere mate, hun derivaten 3- and 15-acetyldeoxynivalenol (3-Ac-DON, 15-Ac-DON), fusarenon X (FusX), neosolaniol (NeoSol) en HT-2 toxine (HT-2). Alle behoren tot de type A en type B trichothecenen, worden geproduceerd door schimmels van het geslacht *Fusarium* en bezitten als basis dezelfde tetracyclische 12,13-epoxytrichotheceen structuur. Door de toegenomen handel in granen, diervoeders en voedingsmiddelen, de wereldwijde contaminatie van deze producten met trichothecenen (en andere mycotoxinen) en het feit dat trichothecenen over het algemeen vrij stabiel zijn en tijdens voedselbereiding niet volledig vernietigd worden, wordt het steeds belangrijker om de eventuele gezondheidsrisico's van de meest voorkomende trichothecenen in kaart te brengen en waar mogelijk toelaatbare dagelijkse innames (TDIs) vast te stellen. Recentelijk is dat door een aantal internationale wetenschappelijke commissies gedaan voor DON, T-2 en HT-2, en dat heeft geresulteerd in (voorlopige) TDIs voor deze stoffen. In dit rapport worden de mogelijke gezondheidseffecten van zes andere trichothecenen, te weten NIV, FusX, DAS, NeoSol en 3- en 15-Ac-DON, onderzocht en wordt getracht ook voor deze trichothecenen (voorlopige) TDIs af te leiden (hetgeen overigens voor NIV ondertussen gedaan is door het Wetenschappelijk Comité voor de Voeding).

In eerste instantie is gekeken naar het voorkomen van de zes trichothecenen in granen, diervoeders en voedingsmiddelen. FusX, DAS, NeoSol en 3- en 15-Ac-DON blijken niet of nauwelijks in deze producten gevonden te worden. Naar alle waarschijnlijkheid zijn deze vijf trichothecenen echter tot nu toe niet routinematig meegenomen geweest in monitoring programma's. NIV wordt daarentegen wel regelmatig aangetroffen, vaak tezamen met DON en zearalenon (ook een *Fusarium* mycotoxine). NIV werd wel gevonden in granen, diervoeders en plantaardige voedingsmiddelen, maar niet in voedingsmiddelen van dierlijke oorsprong.

In tweede instantie is de toxicologie van de zes individuele trichothecenen beschreven, en is een kort overzicht gegeven van de toxicologie van trichothecenen in het algemeen. Alhoewel niet voor alle zes trichothecenen evenveel gegevens beschikbaar waren over hun toxicokinetische en toxische eigenschappen, lijken de effecten gevonden voor NIV, FusX, DAS, 3- en 15-Ac-DON en NeoSol op de effecten zoals die gevonden worden voor trichothecenen in het algemeen. De belangrijkste toxicologische bevindingen in deze evaluatie van de zes trichothecenen bestaan uit haematotoxiciteit, immunotoxiciteit, embryo-

and foetotoxiciteit, braken en verminderde lichaamsgewicht toename en voerconsumptie. Ook lijken sommige een (intrinsiek) genotoxische potentie te hebben.
Voor NIV zijn voldoende gegevens beschikbaar om een voorlopige TDI van 0,7 µg/kg lichaamsgewicht af te kunnen leiden. Deze voorlopige TDI is gebaseerd op de meest gevoelige effecten zoals die voor NIV worden gevonden in proefdieren, namelijk algemene toxiciteit en immunotoxiciteit in muizen. De TDI heeft een voorlopig karakter vanwege het ontbreken van relevante gegevens in de toxicologische dataset.
Voor FusX, DAS en met name voor 3- en 15-Ac-DON en NeoSol zijn de toxicologische datasets zodanig beperkt dat geen TDI (zelfs geen voorlopige) afgeleid kan worden. Om vast te kunnen stellen of deze vijf trichothecenen eventueel een gezondheidsrisico vormen voor mens en dier wordt aanbevolen om in monitoring programma's meer routinematig aandacht te schenken aan FusX, DAS, 3- en 15-Ac-DON en NeoSol. Er zijn analysemethoden voor deze stoffen beschikbaar. Afhankelijk van óf, en de mate waarin, deze vijf trichothecenen worden aangetroffen in granen, diervoeders en voedingsmiddelen, dient vervolgens bezien te worden hoe moet worden omgegaan met de beperkte toxicologische datasets voor deze stoffen.
Omdat de meest voorkomende trichothecenen een overeenkomstige chemische structuur hebben en mogelijk ook een overeenkomstig werkingsmechanisme, verdient het aanbeveling om te bekijken of voor de groep van trichothecenen als geheel een zogenaamde groeps TDI vastgesteld zou moeten worden. Op dit moment zijn daarvoor te weinig gegevens beschikbaar.

Summary

Trichothecenes are an important class of mycotoxins, i.e. naturally occurring, toxic compounds produced by fungi. Of the fungal genera known to produce trichothecenes, *Fusarium* spp. are the most important ones given their global occurrence and the range of trichothecenes they produce. When ending up in animal feed and human foodstuffs, trichothecenes can pose a threat to animal and human health by the induction of toxic syndromes. Only a few of over 150 known trichothecenes are believed to be of importance with respect to their actual presence in crops, feeds and foods. These are deoxynivalenol (DON), nivalenol (NIV), diacetoxyscirpenol (DAS), T-2 toxin (T-2) and, to a lesser extent, their derivatives 3- and 15-acetyldeoxynivalenol (3-Ac-DON, 15-Ac-DON), fusarenon X (FusX), neosolaniol (NeoSol) and HT-2 toxin (HT-2). They belong to the type A and type B trichothecenes and share the same basic chemical structure, a tetracyclic 12,13-epoxy-trichothecene skeleton. Given the worldwide trade in cereals, feeds and foods that more and more are globally contaminated with mycotoxins, including trichothecenes, it has now become important to assess the potential health risks of the most commonly occurring trichothecenes and to derive tolerable daily intakes (TDIs) where possible. Moreover so because trichothecenes are in general stable compounds, and most food processing operations are not successful in complete decontamination.

Recently, the potential risks of DON, T-2 and HT-2 have been assessed by several international committees, which resulted in (temporary) TDIs for these compounds. In this report we therefore address the potential health effects of six other trichothecenes, i.e. NIV, FusX, DAS, NeoSol and 3- and 15-Ac-DON, and try to derive (temporary) TDIs for these trichothecenes as well, although in the mean time an international committee already did that for NIV.

Upon investigation of the occurrence of these six trichothecenes in cereal grains, animal feed and human foodstuffs, it appeared that FusX, DAS, NeoSol and 3- and 15-Ac-DON have rarely been reported to occur in these commodities. It should be noted, though, that in most monitoring programs these five trichothecenes likely have not been routinely included. NIV was reported to occur more frequently, often together with DON and zearalenone, the latter also being a *Fusarium* mycotoxin. NIV was found in cereal grains, in animal feed and in human food of plant-origin (cereals and processed grains) but not in human food derived from animals given contaminated feed.

After reviewing the toxicology of trichothecenes in general, the toxicological data on the six individual trichothecenes subject of this report were addressed. Data on the toxicokinetics and the toxic effects were studied, although the available data package was rather limited for some of the six trichothecenes. Overall, however, the toxic effects observed for NIV, FusX, DAS, 3- and 15-Ac-DON and NeoSol seemed to fit quite well the profile of toxic effects of trichothecenes in general. The most important observed effects were haematotoxicity,

immunotoxicity, embryo- and fetotoxicity, reductions in body weight gain and food consumption, vomiting and, for some, (intrinsic) genotoxicity.
The data available on NIV allowed us to derive a temporary TDI of 0.7 µg/kg bw. This temporary TDI was based on the most sensitive effects of NIV observed in the tested animal species, i.e. general toxicity and immunotoxicity in mice. Due to deficiences in the data set a temporary and not a full TDI was derived.
In our opinion, the limited data available on the toxicology of FusX, DAS and, especially, 3- and 15-Ac-DON and NeoSol, do not allow derivation of a (temporary) TDI for these compounds. In order to assess whether or not these five trichothecenes are a potential threat to animals or humans, it is recommended to pay more attention to FusX, DAS, 3- and 15-Ac-DON and NeoSol in routine monitoring programs. Analytical methods for these compounds are available. When it turns out that these trichothecenes are regularly found at considerable levels in cereal grains, feeds or foods, it should be considered how to deal with the limited data sets on the toxicology of these compounds.
Consideration should also be given to the appropriateness of the derivation of a group TDI for trichothecenes since the most commonly occurring trichothecenes share a common basic chemical structure and, presumably, also a common mechanism of action.

1. Introduction

Many species of *Fusarium*, *Aspergillus, Penicillum* and *Alternaria* are not only recognised plant pathogens but are also sources of mycotoxins. Mycotoxins are naturally occurring, toxic compounds, produced by fungi infecting agricultural crops, particularly cereals and oilseeds. Infection can occur during growth and storage of crops, but also later on in processed foods and feeds. Upon ingestion, these mycotoxins can produce toxic syndromes (mycotoxicoses) in animals and humans.

An important class of mycotoxins are the trichothecenes. Fungal genera known to produce trichothecenes are *Fusarium*, *Trichoderma*, *Trichothecium*, *Stachybotrys*, *Verticimonosporium*, *Cephalosporium*, *Myrothecium*, and *Cylindrocarpon*. The fusaria are by far the most important of these genera because they are widespread globally and produce the greatest range of trichothecenes. However, of over 150 known trichothecenes, only a few appear to be of importance with respect to their actual presence in crops intended for human or animal use. These are: deoxynivalenol (DON; also known as vomitoxin), nivalenol (NIV), diacetoxyscirpenol (DAS) and T-2 toxin (T-2), and, less frequently, certain derivatives (3-acetyldeoxynivalenol (3-Ac-DON), 15-acetyldeoxynivalenol (15-Ac-DON), fusarenon X (FusX), neosolaniol (NeoSol) and HT-2 toxin (HT-2)).

There is increasing evidence of global contamination of cereals and animal feeds with *Fusarium* mycotoxins, and trade in these commodities may contribute to the worldwide dispersal of the mycotoxins. It has therefore become increasingly important to assess the potential health risks associated with exposure to the different *Fusarium* mycotoxins. From the point of view of animal health and productivity the trichothecenes (the focus of this report), zearalenone, moniliformin and the fumonisins are the most important *Fusarium* mycotoxins. However, information to base a risk assessment on is scarce, despite the fact that mycotoxicoses have been known to occur since ancient times.

Although since a few years analytical methods are available for the nine most occurring trichothecenes, up till now in monitoring programs most attention has been focussed on DON. Hence, compared to other trichothecenes, relatively a lot of data are reported on the occurrence of DON in feeds and foods. Also the toxicology of DON is relatively well investigated, like that of T-2 and HT-2. For these three trichothecenes risk assessments have recently been performed by the Scientific Committee on Food (SCF) of the European Commission (SCF, 1999, 2001, 2002), the Joint FAO/WHO Expert Committee on Food Additives (JECFA; WHO/FAO, 2001) and the Nordic Working Group (Eriksen and Alexander, 1998). Each established Tolerable Daily Intakes (TDI) for DON, T-2 and HT-2. Whereas the SCF and Eriksen and Alexander (1998) also evaluated NIV, only the SCF established a TDI for NIV (SCF, 2000, 2002).

For DON, a temporary TDI (tTDI) of 1 µg/kg bw was established by the SCF (SCF, 1999), based on a 2-year feeding study in mice. In this study reduced growth was the most sensitive parameter, with a no-observed-adverse-effect-level (NOAEL) of 0.1 mg/kg bw. An uncertainty factor of 100 was applied to this NOAEL, in order to extrapolate from rodents to humans (factor 10) and to cover for (human) interindividual differences (factor 10). This tTDI would also protect against other toxic effects of DON, including the acute vomiting effect. The tTDI set by the SCF in 1999 is in line with the tTDI established for DON by the Nordic Working Group (Eriksen and Alexander, 1998) and Pieters *et al.* (1999) and with the provisional maximum TDI (PMTDI) established by JECFA (WHO/FAO, 2001). The SCF made the TDI temporary pending a group evaluation. This because DON belongs to a group of trichothecenes sharing a common basic chemical structure and common mechanisms of toxic action and because most *Fusarium* species are capable of producing several trichothecenes. In 2002, after having evaluated four of the most commonly occurring trichothecenes, the SCF performed a group evaluation of DON, NIV, T-2 and HT-2 and considered the appropriateness of a group TDI. SCF concluded that the available data, while limited, did not support the establishing of a group TDI for the four trichothecenes evaluated and, with respect to DON, changed the tTDI of 1 µg/kg bw into a full TDI (SCF, 2002). The SCF, as well as JECFA, expressed the need for further studies.

In 2001, the SCF established a tTDI of 0.06 µg/kg bw for the sum of T-2 and HT-2 (SCF, 2001). This because *in vivo* T-2 is rapidly metabolised to HT-2. Hence, the toxic effects of T-2 and its metabolite HT-2 cannot be differentiated, and the toxicity of T-2 *in vivo* might be due at least partly to HT-2. The tTDI was based on haematotoxic and immunotoxic effects observed in a 3-week feeding study with T-2 in swine. The lowest-observed-adverse-effect-level (LOAEL) for these effects was 0.03 mg/kg bw/day, a dose level at which also reduced feed intake was seen. The uncertainty factor used was 500: an extra uncertainty factor of 5 was applied to account for some deficiencies in the data set and the use of a LOAEL, which was presumably close to the NOAEL. The TDI was made temporary pending the evaluation of the group of trichothecenes as a whole, and also because of gaps in the database. JECFA (WHO/FAO, 2001) came to the same value of 0.06 µg/kg bw as PMTDI for the sum of T-2 and HT-2, and both SCF and JECFA expressed the need for further studies. The Nordic Working Group set a different tTDI of 0.2 µg/kg bw. Because of concern on the seriousness of the possible carcinogenic effect of T-2, they applied an uncertainty factor of 1000 to the highest level without tumourigenic effect (approximately 0.1-0.2 mg/kg bw). This tTDI was also for the sum of T-2 and HT-2 (Eriksen and Alexander, 1998).
After having concluded that the available data, while limited, did not support the establishing of a group TDI for DON, NIV, T-2 and HT-2, the SCF in 2002 confirmed the combined tTDI of 0.06 µg/kg bw for T-2 and HT-2 and recommended that further studies should fill the data gaps (SCF, 2002).

The SCF also recently evaluated the toxicity of NIV. They set a tTDI of 0.7 µg/kg bw based on a LOAEL of 0.7 mg/kg bw/day found in long-term dietary studies with mice. For NIV reduced growth and haematotoxicity/immunotoxicity were the most critical effects. The SCF applied a large uncertainty factor of 1000 because of the use of a LOAEL and the limited database. The TDI was made temporary pending a group evaluation of the trichothecenes, and also because of gaps in the database. A need for further studies was expressed (SCF, 2000). NIV was also evaluated by the Nordic Working Group (Eriksen and Alexander, 1998), but they did not find the available toxicity data sufficient to set a (t)TDI for NIV.
In 2002, the SCF performed a group evaluation of four trichothecenes and concluded that the available data, while limited, did not support the establishing of a group TDI for DON, NIV, T-2 and HT-2. They therefore confirmed the tTDI of 0.7 µg/kg bw for NIV and recommended that further studies should fill the data gaps (SCF, 2002).

When in the near feature other trichothecenes than just DON will be more systematically and routinely included in monitoring programs, more insight is needed in the toxicology of the trichothecenes that up till now only received little or no attention. Hence, in this report we address the potential health effects of NIV, 3-Ac-DON, 15-Ac-DON, DAS, FusX and NeoSol, and try to derive (t)TDIs for these trichothecenes as well.

2. Trichothecenes

2.1 Chemical structure

Trichothecenes are a family of closely related sesquiterpenoids produced by several plant pathogenic fungi. They have a tetracyclic 12,13-epoxytrichothecene skeleton in common, and can be divided into four categories (WHO, 1990):
Type A: characterized by a functional group other than a ketone at C-8
Type B: characterized by a carbonyl function at C-8
Type C: characterized by a second epoxide group at C-7,8 or C-9,10
Type D: characterized by a macrocyclic ring system between C-4 and C-15 with two ester linkages.
The trichothecenes that are subject of this report belong to the type A and type B trichothecenes (see below). Examples of type C trichothecenes are crotocin and baccharin, and of type D trichothecenes roridin A, satratoxin H and verrucarin A.

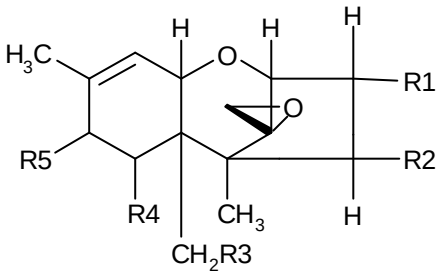

Figure 1 *Structural formula of type A trichothecenes*

Table 1 *Examples of type A trichothecenes*

	Cas nr.	Mol. formula	Mol. wt	R1	R2	R3	R4	R5
DAS [a]	2270-40-8	$C_{19}H_{26}O_7$	366	OH	OAc	OAc	H	H
NeoSol [b]	36519-25-2	$C_{19}H_{26}O_8$	382	OH	OAc	OAc	H	OH
T-2 [c]	21259-20-1	$C_{24}H_{34}O_9$	466	OH	OAc	OAc	H	$OCOCH_2CH(CH_3)_2$
HT-2 [d]	26934-87-2	$C_{22}H_{32}O_8$	424	OH	OH	OAc	H	$OCOCH_2CH(CH_3)_2$

[a] diacetoxyscirpenol (trichothec-9-ene-3,4,15-triol, 12,13-epoxy-, 4,15-diacetate; anguidine)
[b] neosolaniol (trichothec-9-ene-3,4,8,15-tetrol, 12,13-epoxy-, 4,15-diacetate)
[c] T-2 toxin (trichothec-9-ene-3,4,8,15-tetrol, 12,13-epoxy-, 4,15-diacetate 8-(3-methylbutanoate))
[d] HT-2 toxin (trichothec-9-ene-3,4,8,15-tetrol, 12,13-epoxy-, 15-acetate 8-(3-methylbutanoate))

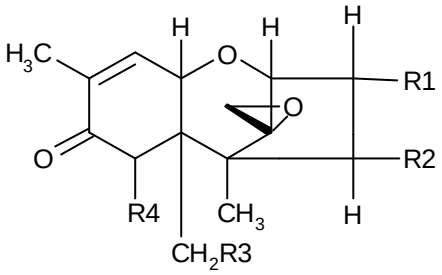

Figure 2 *Structural formula of type B trichothecenes*

Table 2 *Examples of type B trichothecenes*

Name	Cas nr.	Mol. formula	Mol. wt	R1	R2	R3	R4
NIV [a]	23282-20-4	$C_{15}H_{20}O_7$	312	OH	OH	OH	OH
FusX [b]	23255-69-8	$C_{17}H_{22}O_8$	354	OH	OAc	OH	OH
DON [c]	51481-10-8	$C_{15}H_{20}O_6$	296	OH	H	OH	OH
3-Ac-DON [d]	50722-38-8	$C_{17}H_{22}O_7$	338	OAc	H	OH	OH
15-Ac-DON [e]	88337-96-6	$C_{17}H_{22}O_7$	338	OH	H	OAc	OH

[a] nivalenol (trichothec-9-en-8-one, 12,13-epoxy-3,4,7,15-tetrahydroxy-)
[b] fusarenon X (trichothec-9-en-8-one, 4-(acetyloxy)-12,13-epoxy-3,7,15-trihydroxy-; fusarenon; 4-acetyl nivalenol; nivalenol 4-O-acetate; nivalenol monoacetate)
[c] deoxynivalenol (trichothec-9-en-8-one, 12,13-epoxy-3,7,15-trihydroxy-; dehydronivalenol; 4-deoxynivalenol; Rd toxin; vomitoxin)
[d] 3-acetyl-deoxynivalenol (trichothec-9-en-8-one, 3-(acetyloxy)-12,13-epoxy-7,15-dihydroxy-)
[e] 15-acetyl-deoxynivalenol (trichothec-9-en-8-one, 15-(acetyloxy)-12,13-epoxy-3,7-dihydroxy-)

2.2 Physical and chemical properties

The trichothecenes are colourless, mostly crystalline solids. Type A trichothecenes are soluble in moderately polar solvents, such as chloroform, diethyl ether, ethyl acetate, and acetone, whereas type B trichothecenes require higher polarity solvents, such as aqueous methanol or aqueous acetonitrile.
Type A and B trichothecenes lack conjugated unsaturation in their structures with a consequent absence of UV absorption, except for end absorption due to unsaturation at C9-C10. They also exhibit no fluorescence under UV light.

When trichothecenes containing an ester group are treated with a base, they are hydrolysed to their corresponding parent alcohol. Free hydroxyl groups are readily acylated.

The 12,13-epoxy group is extremely stable to nucleophilic attack. However, prolonged heating under highly acidic conditions causes an intramolecular rearrangement of the trichothecene skeleton to the apotrichothecene ring.

The trichothecenes are generally stable. They remain unaffected when refluxed with various organic solvents and also under mildly acidic conditions (WHO, 1990; Ueno, 1987b).

2.3 Analysis of trichothecenes

Langseth and Rundberget (1998) and Krska *et al.* (2001) amongst many others, provided exhaustive reviews of quantitative and qualitative methods for the determination of trichothecenes.
In general four major steps are necessary for the determination of trichothecenes; sampling (including sample preparation), extraction, clean-up and detection and quantification of the toxins.

2.3.1 Sampling

In general, mycotoxins are inhomogeneously distributed in the commodities to be inspected, which makes it difficult to obtain a representative sample (Van Egmond and Speijers, 1999). To reduce the variance, clearly defined sampling plans are required, including in general large sample sizes. To prepare a representative test portion for analysis, the sample is ground and thoroughly mixed.

2.3.2 Extraction

Test portions of 20 to 50 g of sample are normally used for extraction. Lower quantities can be used, but require great care in order to obtain a homogeneous test portion.
Various combinations of solvents have been used for the extraction of the trichothecenes from the test portion, including methanol-water, acetonitrile-water and ethyl acetate-acetonitrile in various ratios (Mateo *et al.*, 2001).
Nowadays acetonitrile/water (84+16, v/v) is the most extensively used extraction medium for trichothecene analysis (Langseth and Rundberget, 1998).
The extraction time is strongly dependent on the particle size, the used shaker and extraction flask and has to be optimised for every combination.

2.3.3 Clean-up

Various procedures for clean-up of the extracts have been published. Most procedures include purification of the extract by means of columns packed with adsorbents like silica,

Florisil® or charcoal-alumina. A modified charcoal-alumina based column, the MycoSep® column, is commercially available and showed very good results (Radová *et al.*, 1998; Weingaertner *et al.*, 1997).
Recently immunoaffinity columns for DON and T-2/HT-2 became commercially available. (Cahill *et al.*, 1999). Drawbacks are that these columns are only applicable for a single toxin and that recoveries in general are poor.

2.3.4 Detection and quantification

Methods that have been applied to identify and quantify the trichothecenes are

- Thin-layer chromatography (TLC),
- High-performance liquid chromatography (HPLC) with post or pre-column derivatization and either UV detection, fluorescence detection (FLD) or mass spectrometric (MS) detection,
- Supercritical fluid chromatography (SFC),
- Capillary gas chromatography (GC) with either electron-capture detection (ECD), flame ionisation detection (FID) or mass spectrometric (MS) detection (Schothorst and Jekel, 2001).

At present, gas chromatographic methods with ECD or MS detection are most commonly used (Langseth and Rundberget, 1998).
Prior to GC analysis, trichothecenes require derivatization of the hydroxyl groups, which can be performed with a number of different agents. The choice of derivatizing agent depends on the trichothecene analysed and detection method used.
For screening purposes enzyme-linked immunosorbent assays (ELISA) (Krska *et al.*, 2001) can be used. In general, no clean-up is required after extraction of the mycotoxin. The assay can therefore be applied directly to the crude extract and the results are quickly available. ELISA methods are very sensitive, however the uncertainty of the results is in general high. ELISA methods are not available for all trichothecenes.

In monitoring programs in the Netherlands on the presence of trichothecenes in wheat, the GC-FID method is used. In short, the procedure involves extraction of trichothecenes from the sample matrix by acetonitrile/water (84/16, v/v). Then, two different Mycosep® clean-up columns are used to purify the extract. The extract is evaporated to dryness and the trichothecenes are derivatised to trimethylsilyl (TMS) ethers at room temperature. The residue is dissolved in iso-octane and washed with water. The final extract is analysed for trichothecenes by GC with FID. Quantification is based on the internal standard α-chloralose. This GC-FID method produced good results in an intercomparison study of trichothecene analysis within the European Union Standards, Measurements and Testing Programme (Schothorst and Jekel, 2001).

3. Sources and occurrence of trichothecenes

3.1 Sources

There are several fungal genera known to produce trichothecenes: *Fusarium, Trichoderma, Trichothecium, Stachybotrys, Verticimonosporium, Cephalosporium, Myrothecium, Cylindrocarpon*. The fusaria are by far the most important of these genera because they are widespread globally and produce the greatest range of trichothecenes. Production of trichothecenes depends on many factors, including substrate, temperature, humidity etc. Besides, within one fungal species more than one toxin can be produced (depending on the strain and/or environmental conditions), while a certain trichothecene can be produced by different fungal strains/species. The identification of trichothecene-producing fusaria is complex because of the existence of different taxonomic systems, but *F. sporotrichioides* and *F. graminearum* (*Gibberella zeae* in perfect stage) are generally considered very important in trichothecene mycotoxicology. *F. sporotrichioides* (synonym of *F. tricinctum*) is mainly associated with the production of type A trichothecenes (T-2, HT-2, DAS, NeoSol and related trichothecenes), while *F. graminearum* is the major producer of NIV, DON and related trichothecenes (see also Table 3 below). There are two chemotypes of *F. graminearum*, one producing NIV, FusX and 4,15-diacetyl-NIV (NIV type) and the other producing DON, 3- and 15-Ac-DON and 3,15-diacetyl-DON (DON type) (Ichinoe and Kurata, 1983; Ueno, 1987a/b; WHO, 1990; IARC, 1993; Smith *et al.*, 1994; Bottalico, 1998).

Table 3 Trichothecene production by Fusarium species

Type A	
T-2, HT-2	*F. sporotrichoioides, F. acuminatum, F. poae*
DAS	*F. poae, F. equiseti, F. sambucinum, F. sporotrichioides, F. acuminatum*
NeoSol	*F. sporotrichioides, F. poae, F. acuminatum, F. equiseti*
Type B	
DON	*F. graminearum, F. culmorum*
NIV	*F. crookwellense, F. poae, F. graminearum, F. culmorum, F. nivale**, *F. equiseti*
FusX	*F. crookwellense, F. poae, F. graminearum, F. culmorum, F. nivale, F. equiseti*
3-Ac-DON	*F. graminearum, F. culmorum*
15-Ac-DON	*F. graminearum, F. culmorum*

* atypical strain of *F. sporotrichioides*

3.2 Plant pathogenicity

The fusaria are considered to be field fungi, since they are primarily plant pathogens. However, if the environmental conditions are favourable they can continue to grow on crops held in storage. Mycotoxin-producing strains of *Fusarium* are a world-wide problem. They have been isolated from Norway's Arctic region, Argentina, Austria, Bulgaria, Canada, China, France, Germany, Greece, Hungary, Italy, Japan, Korea, Nepal, Poland, Portugal, Russia, Sweden, UK, Yemen, Spain, Netherlands, Brazil, Finland, Egypt, Nigeria, India, Malaysia, South Africa, Australia and New Zealand, hence in both temperate and semitropical areas. The fusaria can contaminate a wide range of agricultural crops, especially cereals, for which *Fusarium* species are major pathogens. They infect small grain cereals (wheat, barley, oats, rye, rice, sorghum, millet) and maize, and cause a number of frequently encountered plant diseases (wilt, blight/scab, rot), with severe reductions in crop yield. Certain strains are also capable of producing mycotoxins which can be formed in preharvest infected plants still standing in the fields, or in stored grains. Head blight (scab) of small cereals and ear rot in maize are of the greatest concern, the more so as maize, wheat and barley constitute almost two-thirds of the world production of cereals and almost 80% of the European grain production. *Fusarium graminearum* and *F. culmorum* are the species predominantly found associated with these diseases. When environmental factors such as temperature and humidity are favourable, these fungi produce mycotoxins (especially DON and NIV), which can end up as contaminants in animal feed and food (WHO, 1990; Smith *et al.*, 1994; Bottalico, 1998).
Besides cereals, mycotoxin-producing *Fusarium* species can infect other growing crops, grass, hay and straw, all of which are sources of animal fodder and silage. At harvest or when directly consumed by animals these forage crops could give rise to mycotoxicoses. Upon storage of forage crops, most mycotoxins present will remain stable under aerobic conditions, but in time the field-derived fungi (like *Fusarium*) will be replaced by storage fungi (like *Aspergillus* and *Penicillium*), particularly with inadequate drying or if the moisture content is not maintained below about 15%. Also in silage, *Fusarium* species are not major contaminants because most are aerobic and are unable to grow under the anaerobic conditions used in silage making (Scudamore and Livesey, 1998).

3.3 Contamination of cereal grains and animal feed

An overview of the occurrence of some of the most important *Fusarium* mycotoxins in cereal grains in European countries and worldwide (DON, DAS, NIV, T-2, fumonisin B1, moniliformin and zearalenone) was compiled by Bottalico (1998), utilizing mainly the reviews by Smith *et al.* (1994) and Eriksen and Alexander (1998). In Smith *et al.* (1994), also data on some other trichothecenes subject of this report were presented.

Table 4 Occurrence of Fusarium mycotoxins in cereal grains

	Worldwide	Europe	Worldwide	Europe
	[no. positive/assayed samples (%)]		*(mg/kg)*	
DON [1]	3491/7369 (47)	3771/6288 (60)	1-67000	4-67000
DAS [1]	126/2103 (6)	49/1535 (3)	1-31500	20-31500
NIV [1]	867/2181 (40)	745/4608 (16)	2-37900	3-7800
T-2 [1]	350/4656 (8)	519/4383 (12)	1-38890	1-14000
fumonisin B1 [1]	365/497 (73)	154/179 (86)	1-117520	50-4670
moniliformin [1]	102/288 (35)	37/148 (25)	50-11570	50-750
zearalenone [1]	2277/18018 (13)	845/5745 (15)	2-275800	1-175000
15-Ac-DON [2]	36/70 (51)	-	44-7900	-
3-Ac-DON [2]	128/545 (23)	1/33 (3)	5-1900	50
FusX [2]	10/379 (3)	0/199 (0)	40-1000	-
NeoSol [2]	4/393 (1)	2/306 (1)	100-400	300-400

Source: [1] Bottalico (1998), [2] Smith *et al.* (1994)

From a review by Placinta *et al.* (1999) it appears that besides cereal grains also animal feed is contaminated worldwide with *Fusarium* mycotoxins (see Table 5). This is not surprising, since cereals, like forage crops, are important sources of animal fodder and silage. Contamination is, to a significant degree, linked with specific cereal diseases caused by *Fusarium* pathogens. Trade in these commodities may contribute to the worldwide dispersal of mycotoxins. Of the *Fusarium* mycotoxins, DON, NIV, zearalenone and the fumonisins are of major concern, because of their ubiquitous distribution and effects on animal health. Concentrations of NIV and zearalenone in grains are generally low relative to those for DON (see Table 5).

The data for the Netherlands mentioned in the review by Placinta *et al.* (1999) originate from a 1990 survey. More recent surveys into the occurrence of trichothecenes in imported wheat in the Netherlands showed the presence of DON in 19 out of 22 harvest samples from the year 1998 at levels ranging from 76-1654 µg/kg. In none of these 1998 harvest samples NIV, FusX, DAS, NeoSol, 3-Ac-DON, T-2 or HT-2 were detected (limit of quantification 75 µg/kg) (Schothorst and Jekel, 2001). In harvest samples from the year 1999 more or less the same findings were observed, with DON present in 21 out of 25 samples at levels ranging from 81-2477 µg/kg. Furthermore, NIV was detected in 2 out of 25 samples at levels of 53 and 150 µg/kg. Other trichothecenes (FusX, DAS, NeoSol, 3-Ac-DON, T-2 and HT-2) were not found above the limit of quantification of 75 µg/kg (Schothorst, 2000).

Table 5 Global distribution of DON, NIV and zearalenone in cereal grains and animal feed

		DON	NIV	Zearalenone
		(mg/kg)		
Germany	Wheat	0.004-20.5	0.003-0.032	0.001-8.04
Poland	Wheat	2-40	0.01	0.01-2
Poland	Maize kernels	4-320		
	Maize cobs: axial stems	9-927		
Bulgaria	Wheat	up to 1.8		up to 0.12
Finland	Feeds and grains	0.007-0.3		0.022-0.095
	Oats	1.3-2.6		
Norway	Wheat	0.45-4.3	max 0.054	
	Barley	2.2-13.33	max 0.77	
	Oats	7.2-62.05	max 0.67	
Netherlands	Wheat	0.020-0.231	0.007-0.203	0.002-0.174
	Barley	0.004-0.152	0.030-0.145	0.004-0.009
	Oats	0.056-0.147	0.017-0.039	0.016-0.029
	Rye	0.008-0.384	0.010-0.034	0.011
South Africa	Maize	up to 1.83	up to 0.37	
South Africa	Cereals/animal feed			0.05-8.0
India	*Paspalum palidosum*			
	Straw			0.422
	Mixed concentrate			0.843
Philippines	Maize		0.018-0.102	0.059-0.505
Thailand	Maize			0.923
Korea	Barley	0.005-0.361	0.040-2.038	
	Maize	mean 0.145	mean 0.168	
Vietnam	Maize powder	1.53-6.51	0.78-1.95	
China	Maize	0.49-3.10	0.6	
Japan	Wheat	0.03-1.28	0.04-1.22	0.002-0.025
	Barley			0.010-0.658
Japan	Wheat	0.029-11.7	0.01-4.4	0.053-0.51
	Barley	61-71	14-26	11-15
New Zealand	Maize	max 3.4-8.5	max 4.4-7.0	max 2.7-10.5
USA	Wheat	up to 9.3		
USA	Wheat (winter), 1991	<0.1-4.9		
	Wheat (spring), 1991	<0.1-0.9		
	Wheat, 1993	<0.5-18		
	Barley, 1993	<0.5-26		
Canada	Wheat and barley	up to 0.5		up to 0.3
Canada	Wheat (hard)	0.01-10.5		
	Wheat (soft, winter)	0.01-5.67		
	Wheat (soft, spring)	0.01-1.51		
	Maize	0.02-4.09		
Canada	Animal feeds	0.013-0.2	0.065-0.311	
Argentina	Wheat	0.10-9.25		
Brazil	Wheat	0.47-0.59	0.16-0.40	0.04-0.21

Source: Placinta *et al.*, 1999

There is often co-occurrence of several *Fusarium* mycotoxins in the same sample of grain or animal feed. DON and NIV regularly co-occur, but also other type A and/or type B trichothecenes (like 3-Ac-DON, T-2, HT-2, 15-Ac-DON, DAS or FusX) or zearalenone/fumonisins might occur next to DON and/or NIV. This multiple contamination is of particular concern, moreover because it is often in the presence of aflatoxin B1, one of the most notorious mycotoxins (Placinta *et al.*, 1999).

WHO (1990) and IARC (1993) also reported the wordwide occurrence of trichothecenes in cereals and animal feed, with clear regional and within-regional differences. T-2, DON and NIV are amongst the most frequently found trichothecenes, with DON being the most widely distributed *Fusarium* mycotoxin, often occurring with NIV as co-contaminant. Occasionally other trichothecenes (like DAS and 3- and 15-Ac-DON) have been found in agricultural products (mostly corn). Small amounts of FusX, the acetylated precursor of NIV, can occur together with NIV. FusX hardly ever occurs in cereals, and if found, it is together with other *Fusarium* toxins produced by the same fungal species (NIV and zearalenone).

3.4 Contamination of human foodstuffs

Although of all known trichothecenes only a few appear to be of importance with respect to their actual presence in crops and animal feed (especially DON, NIV, T-2 and DAS, and to a lesser extent certain derivatives thereof), they can cause problems to humans when ending up in human foodstuffs. These foodstuffs can be of plant-origin (cereals and processed grains) or of animal-origin (residues in meat, milk, eggs of animals given contaminated feed). The vast majority of the confirmed cases of contamination of plant-based foodstuffs by trichothecenes involve DON in wheat or wheat products (Table 6), overall at concentrations <1 mg/kg, but occasionally also well above 1 mg/kg. NIV has also been detected (particularly in Japan), but other type A or B trichothecenes have hardly been found. Frequently, there is concurrent exposure to more than one *Fusarium* mycotoxin (often DON and NIV, along with zearalenone) (Pohland and Wood, 1987; WHO, 1990).

Table 6 Global occurrence of trichothecenes in human food

		DON	NIV	Zearalenone
		[mg/kg (no.positive/total)]		
Canada	cornflakes			13-20 (1/1)
USA	corn meal			11-69 (9/10)
France	walnuts			50-450 (3/60)
	sweetcorn	20000-500000 (33/33)		400-270000 (7/29)
South Africa	corn [a]	trace-820 (14/36)	trace-240 (6/36)	20 (1/36)
Taiwan	corn (USA) [b]	95-312 (7/100)		49-303 (7/73)
	corn (South Africa)	140 (1/5)		503 (1/8)

		DON	NIV	Zearalenone
		[mg/kg (no.positive/total)]		
Canada	potatoes	- (4/17)		
Canada	wheat	20-1320 (55/199)		
Canada	wheat breakfast cereals	86* (36#)		
	wheat flour	400* (43#)		
	bran	170* (14#)		
	bread (buns)	80* (21#)		
	cookies (biscuits)	120* (35#)		
	crackers	270* (20#)		
	baby cereals (mixed)	43* (30#)		
	corn meal	110* (35#)		
	corn flour	180* (27#)		
	corn syrup/starch	-		
	rye	100 (8#)		
	rye flour	120 (3#)		
	rye bread	58 (4#)		
Japan	parched-barley	27-85 (6#)		
	flours		37-190 (6#)	
Japan	wheat flour	2-239 (26/36)	4-84 (12/36)	1-6 (3/27)
	pressed barley	3-50 (10/14)	8-380 (13/14)	6 (1/3)
	pearled barley	48 (1/1)	220 (1/1)	4 (1/1)
	barley flour	8-39 (3/6)	13-41 (6/6)	1-4 (6/6)
	popcorn (USA)	12-250 (7/7)	- (0/7)	
	Job's-tears	48-496 (2/12)	3-920 (11/12)	10-440 (7/7)
USA	corn meal	nd-250 (45/50)		
	wheat flour	nd-460 (44/50)		
	bread	nd-240 (20/25)		
	snack foods	nd-450 25/44)		
	baby foods	nd-90 (14/39)		
	breakfast cereals	nd-530 (35/60)		
	breakfast cereals	100* (36/60)		
	corn syrup	- (0/9)		
	beer	- (0/14)		
USA	shelled corn	trace-500 (53/96)		
		500-1000 (10/96)		
		1000-2000 (1/96)		
	wheat	trace-500 (38/123)		
		500-1000 (32/123)		
		1000-2000 (4/123)		
		>2000 (1/123)		

Source: Pohland and Wood, 1987

[a] also contained moniliformin (trace-12000 µg/kg (15/36)); [b] also contained T-2 (78-650 µg/kg (9/118)); * average concentration; # total number of products analysed; nd = none detected

Some recent data on the occurrence of DON in plant-based foodstuffs in the Netherlands are given in Table 7.

Table 7 Occurrence of DON in human food in the Netherlands

	Year	DON
		[mean/max in mg/kg (no.positive/total)]
Wheat products	1999	134/250 (18/20)
Wheat	1999	358/1900 (50/54)
Wheat flour	1999	193/460 (20/24)
Maize and by-products	1999	110/130 (3/3)
Malt	1999	- (0/2)
Various grains and by-products	2000	55/570 (80/171)
Various grains (not wheat)	2000	40/385 (5/25)

Source: WHO/FAO, 2001

In contrast to plant-based foodstuffs, there is almost no carry-over of trichothecenes into food of animal origin (meat and edible tissues, milk, eggs). Data available mostly concern DON, and indicate that either no or only very low residual amounts of DON can be detected in tissues of poultry and swine, in eggs and in milk. DON is not transferred into milk from cattle due to de-epoxidation of DON by rumen microorganisms. This might result in low levels of de-epoxy-DON in milk. Due to the fast and extensive metabolism and excretion it is likely that DON and related trichothecenes will not accumulate in the exposed animals, and that transfer into animal-derived products will be marginal (Prelusky, 1994; Spahr *et al.*, 1999). Indeed, in a recent investigation in Germany, no DON or other type A and B trichothecenes were detected in food from cattle, pigs and poultry. It was concluded that trichothecenes are not important as possible contaminants in food from animal origin and as such are not of practical relevance from the view of food hygiene and food safety (Gareis and Wolff, 2000).

3.5 Fate during processing

The data that are available on the influence of food processing operations on the stability of trichothecenes mostly concern DON. Cleaning and milling of wheat are not effective in completely removing DON, which concentrates in the bran. The milling of corn also has little effect on DON content. In dry-milling of corn, DON concentrates in the germ meal, which is used primarily for animal feed. In wet-milling, DON concentrates in the steep liquor but small amounts are also retained in the starch. Baking does not result in DON-free products: depending on the baking conditions, a reduction of 0-49% in DON-content has been observed

(Scott, 1984). However, when baking yeast-containing products, the DON-content increased, which was attributed to enzymatic conversion of DON precursors (Charmley and Prelusky, 1994; Smith *et al.*, 1994). In baking Japanese bread, a reduction of 33-60% was reported for trichothecenes other than DON (not specified). On cooking wheat flour into Japanese noodles a loss of 32-59% of trichothecenes (not specified) was observed, while losses were 68-100% for Chinese noodles. In the latter process, sodium carbonate was used as food additive (Scott, 1984). During tortilla fabrication, at which corn is first boiled in calcium hydroxide, DON and 15-Ac-DON were not stable: only 18-28% of DON was retained, while 15-Ac-DON was destroyed completely (Abbas *et al.*, 1988).

In heat stability experiments Kamimura *et al.* (1987) found that trichothecenes of the NIV- and T-2 type decomposed with increasing temperature, but not completely. A combination of physical and chemical treatment has been reported to be effective in reducing DAS in animal feed: upon treatment with calcium hydroxide monomethylamine, a greater reduction was seen at increased temperatures and moisture content (Bauer *et al.*, 1987).

The use of gamma-irradiation to destroy preformed toxins present in grain does not appear to be a suitable method for detoxification of low moisture grains contaminated with DON and 3-Ac-DON. The high dose required for their destruction on maize (>50 kGy) makes irradiation unsuitable for practical use (O'Neill *et al.*, 1993).

As contaminated grain may contain a wide variety of mycotoxins of differing chemical characteristics (including heat stability, solubility and adsorbent affinity), Charmley and Prelusky (1994) concluded that it is difficult to develop a decontamination method (either physical, chemical or biological, or a combination) that will be equally effective against each mycotoxin present. Moreover, this method should also be reliable, cost-effective and commercially applicable, constituting an extra problem.

4. Toxicology

To put the available information on the toxicology of the six trichothecenes subject of this report into perspective, first an overview on trichothecenes in general is given in chapter 4.1. This overview is a short compilation of data published in a number of reviews and books (a.o. Ueno 1983a; Ueno, 1987a/b; Hsieh, 1987; WHO, 1990; IARC, 1993; Smith *et al.*, 1994; Bondy and Pestka, 2000). Data specific for NIV, FusX, DAS, 3-Ac-DON, 15-Ac-DON and NeoSol are presented in chapters 4.2 - 4.7.

4.1 Trichothecenes in general

4.1.1 Toxicokinetics

Most data available on the toxicokinetics of trichothecenes concern T-2 and, to a lesser degree, DON and DAS. In general, trichothecenes are well absorbed from the digestive tract after which they are widely distributed in many tissues and organs. The main metabolic pathways are conjugation and de-epoxidation, while for some trichothecenes also deacetylation and hydroxylation play a role. For deacetylation the substituents at C3 and C8 play an important role. For example, for deacetylation at C4 it is essential that there is an hydroxyl group at C3 and not an acetyl group, while at the same time the substituent at C8 should be of hydrophobic (acyl, H or =O) in stead of hydrophylic nature (OH).
The metabolites produced are generally less toxic than the corresponding parent toxins.

De-epoxidation is the most important step in the detoxification of trichothecenes. It is carried out by microorganisms in the gastrointestinal tract, and in cattle also by the ruminal flora. The ability to de-epoxidate trichothecenes can be acquired either by an increase in the number of certain microorganisms or an increase in their capacity to metabolise trichothecenes. Trichothecenes have been shown to change the composition of the gastrointestinal microflora (Tenk *et al.*, 1982). It can take a few days before the gastrointestinal flora is adapted to form de-epoxide metabolites. The initial inability to de-epoxidate trichothecenes might be an explanation for the higher toxic effects often found at the start of feeding trichothecenes. Some animal species (amongst which chickens) lack the necessary microflora for epoxide reduction, and they are likely to be more sensitive to trichothecenes.

4.1.2 Toxicity

4.1.2.1 Toxic effects and mode of action

Trichothecene compounds produce a variety of toxic symptoms in humans and animals, including mortality, skin and gastrointestinal irritation or necrosis, haematological disorders

(a.o. initial leucocytosis followed by leukopenia), diarrhoea, vomiting and feed refusal, decreased body weight gain, damage to the haematopoietic systems in bone marrow, spleen, thymus and lymph nodes, and immunological alterations. As to the latter, trichothecenes can both suppress and stimulate immune function, resulting in either impaired resistance to infection or neoplasia, or in hypersensitivity or auto-immune like disorders.

Biochemically, trichothecenes are highly toxic at the subcellular, cellular and organ level. They are very cytotoxic to eukaryotic cells, causing cell lysis and inhibition of mitosis. Trichothecenes are also very potent inhibitors of protein and DNA and RNA synthesis, and they can interact with the cell membrane. Toxicity at the subcellular level is largely due to their ability to inhibit protein synthesis and to covalently bond to sulphydryl groups.
The mechanism of protein inhibition can be of two types. One is inhibition of the initial step of protein synthesis (I-type; examples are T-2, HT-2, DAS, NIV and FusX) and the other inhibition of the elongation-termination step (ET-type; example is DON). It is considered that peptidyl transferase is inhibited with subsequent inhibition of peptide bond formation. The target organell of trichothecene action is the 60S subunit of eukaryotic ribosomes, the protein inhibition activity correlating well with ribosome affinity.
The inhibition of DNA and RNA synthesis by trichothecenes require higher toxin concentrations than the inhibition of protein synthesis and the extent of inhibition is much less. The suppression of nucleic acid synthesis is, however, not simply a secondary effect of the inhibition of protein synthesis. It is assumed that damage to membrane structure and cytoskeleton components affect macromolecule synthesis.

Being potent inhibitors of protein and DNA and RNA synthesis, trichothecenes are especially toxic to tissues with a high cell division rate. As the character of trichothecene-induced lesions (necrosis, karyorrhexis) in actively dividing cells of thymus, spleen, bone marrow, ovary, testes, lymph nodes and intestinal mucosa is very similar to those induced by radiation, trichothecenes are classed as radiomimetic substances.

Given the above, leucocytes and the immune system are the primary target for trichothecenes. *In vitro* and *in vivo* studies have demonstrated that trichothecenes can affect leucocytes by deregulating cytokine production and by inducing apoptosis. The former is induced by exposure to low levels of trichothecenes, with immune stimulation as net effect. Higher levels of trichothecenes promote apoptosis, with immune suppression as net effect.

4.1.2.2 Structure-activity relationships

The minimum structural feature required for biological activity of the trichothecenes is the presence of the 12,13-epoxytrichothecene skeleton. Reduction of the epoxide leads to inactive derivatives, while hydrogenation of 9,10-double bonds and rearrangement of the trichothecene skeleton to the apotrichothecene ring system lead to a substantial loss of activity. The biological activity of trichothecenes is also affected by the nature of the side chains at specific positions.

Some examples of the cytotoxic and biochemical properties of trichothecenes are given in Table 8. Although there is not always a good match between the relative toxicity *in vitro* and *in vivo*, the potency ranking *in vitro* and *in vivo* is more or less the same. The potency of the trichothecenes depends on the modifications of the side chains in the molecule. In general, the macrocyclic trichothecenes (type D) are the most potent, followed by type A, type B and type C trichothecenes. Within the type A trichothecenes, substances with an acetyl group at R3 are the most potent. Removal of this acetyl group results in a pronounced decrease in potency, while removal of the acetyl group at R2 and the sidechain at R5 result in a smaller loss of activity. In contrast, addition of an acetyl group at R1 also results in a loss of potency. Within the type B trichothecenes, the potency is mainly influenced by the substituent at R2, the potency decreasing in the order from acetyl > hydroxyl > hydrogen. Also for type B trichothecenes, addition of an acetyl group at R1 results in a loss of potency (Thompson and Wannemacher, 1986; Eriksen and Alexander, 1998).

Table 8 *Cytotoxicity and protein synthesis inhibition of trichothecenes in cultured human [a] and animal cells*

	HeLa [1]	HEK [1]	HL [1]	HEp2 [1]	Rabbit reti-culocytes [1]	Vero cells [b,2]	Rat spleen lymphocytes [2]
	(ID_{50} in mg/ml)					*[ID_{50} in nM (relative potency [c])]*	
Type A							
T-2	0.01	0.02	0.003	0.001	0.03	14 (100)	6 (100)
HT-2	0.01	0.1	0.01		0.03	65 (22)	10 (63)
DAS	0.01	0.01	0.001		0.03	27 (53)	12 (53)
NeoSol	0.1	0.06	0.05		0.25	273 (5.2)	127 (4.8)
Type B							
DON	1.0	3.0	0.5	0.250	2.0	1499 (0.95)	850 (0.72)
NIV	0.3	1.0	0.3	0.225	3.0	8131 (0.18)	6835 (0.089)
FusX	0.1	1.0	0.3		0.25	288 (5.0)	91 (6.7)
3-Ac-DON	10	10	10		10	26279 (0.054)	4293 (0.14)
15-Ac-DON	---------- no data available ----------						
Type C							
Crotocin	0.5	0.6	2.0	0.25	1		
Type D							
verrucarin A	0.005	0.002	0.003	0.001	0.01	12 (118)	3 (191)
roridin A	0.003	0.003	0.003		0.01	12 (117)	5 (115)

[a] origin: uterine carcinoma (HeLa), embryonic kidney (HEK), lymphocytes (HL), epidermoid carcinoma (HEp2); [b] origin: monkey kidney; [c] compared to T-2, set as standard having 100% potency
Source: [1] Ueno (1983a,b), [2] Thompson and Wannemacher (1986)

Trichothecenes inhibit the protein synthesis by binding to the ribosomes according to the substituents at C3 (R1) and C4 (R2). Trichothecenes with substituents at both C3 and C4 inhibit mainly polypeptide chain initiation while trichothecenes lacking substituents at one or both positions mainly inhibit elongation (Eriksen and Alexander, 1998).

4.1.2.3 Animal and human mycotoxicoses

For many years, *Fusarium* species have been known to be associated with a number of human and animal toxicoses. However, only rarely a direct connection with the mycotoxin(s) involved has been established factually.

Animal mycotoxicoses associated with trichothecene-producing *Fusarium* species include amongst others the haemorrhagic syndrome (*F. sporotrichioides* and *F. poae)*, Akakabi-byo (red mould disease or scabby grain intoxication; *F. graminearum*), feed refusal and emetic syndromes (*F. graminearum*), ill-thrift, oral and other gastrointestinal lesions. Natural outbreaks have been reported to occur all over the world, thereby severely compromising livestock health, welfare and productivity (Nelson *et al.*, 1994; D'Mello *et al.*, 1999).

Mycotoxin outbreaks affecting humans have mainly been reported to occur in the former Soviet Union and Asia (Japan, China, Korea), both historically and more recently. Historical outbreaks associated with *Fusarium* species include alimentary toxic aleukia (*F. sporotrichioides* and *F. poae*; closely related to the haemorrhagic syndrome in animals), Urov or Kashin-Beck disease (*F. poae*) and Akakabi-byo (*F. graminearum*). Although there is a strong suspicion of involvement of certain type A (T-2, DAS) and/or type B trichothecenes (DON, NIV, FusX) in these diseases, none has been positively identified. More recent outbreaks occurred in China and in India, in total affecting thousands of people. The mouldy cereals causing poisoning in China in 1984/5 contained DON and zearalenone but no T-2 or NIV. Symptoms included nausea, vomiting, abdominal pain, diarrhoea, dizziness and headache; no death occurred. In 1987 in India, bread made from mouldy flour caused intoxication (abdominal pain, throat irritation, diarrhoea, blood in stools and vomiting; no fatalities occurred). The mouldy flour contained DON, Ac-DON (not specified), NIV and T-2.
In corn and in wheat and barley samples implicated in two poisoning outbreaks in China in 1989 and 1991, DON was the predominant toxin, followed by zearalenone and NIV. 3-Ac-DON and 15-Ac-DON were only detectable in highly contaminated wheat and corn, respectively. Corn contained also fumonisins. FusX, T-2 and HT-2 were not present. In the 1991 outbreak about 130000 people were affected by gastrointestinal disorders, including abdominal pain and fullness, nausea, vomiting, fatigue and fever (Li *et al.*, 1999).

IARC (1993) described studies addressing the relationship between exposure to *Fusarium* toxins and oesophageal cancer in the Transkei (South Africa) and in Linxian (China). Most studies pointed to a co-existence of a mixture of toxins from several *Fusarium* species (DON, zearalenone, NIV, 3-Ac-DON and/or 15-Ac-DON) on maize, the main dietary staple.

According to IARC the studies suggested no correlation with the above mentioned trichothecenes.

4.1.2.4 Co-exposure

Cereal grains, animal feed and human foodstuffs are quite often contaminated with more than one mycotoxin, not only derived from *Fusarium* fungi but also from other fungi like for example *Aspergilli*. Hence, in practice exposure of animals and humans will be to a mixture of mycotoxins. Research, however, has mainly concentrated on the toxicity of some single pure mycotoxins. Combined effects (antagonistic, additive and/or synergistic) of two or more mycotoxins, as well as toxicological effects at the low levels as usually found in contaminated feed and food, have received little attention.

4.2 Nivalenol (NIV)

4.2.1 Toxicokinetics

4.2.1.1 Absorption, distribution and excretion

Within 72 hrs after a single oral dose of 5 mg NIV/kg bw to male rats, 24% of the dose was excreted as NIV and de-epoxy-NIV via faeces and 15.5% via urine. In serum, liver and kidney the levels of NIV were lower than in urine or faeces even 1 h after dosing, and they were rapidly lowered. No de-epoxy-nivalenol was detected in serum, liver or kidney within 24 hrs after dosing (Onji, 1990).
After repeated oral administration of 5 mg NIV/kg bw to male Wistar rats (12 times at 2- or 3-day intervals), excretion was also mainly via faeces (>87%) (Onji *et al.*, 1989).

In male swine given NIV in the diet at a dose of 0.05 mg/kg bw, twice daily for three days, NIV was already detected in blood samples taken 20 min after the start of feeding. During the first 7.5 hrs, 11-43% of the NIV dose was absorbed. Blood peak concentrations of 3-6 ng NIV/ml were reached within 2.5-4.5 h after feeding. Sixteen hours after feeding, NIV was still being absorbed from the intestine, with blood concentrations of 1-3 ng NIV/ml. Hence, absorption was rapid and extensive. NIV was mainly excreted in faeces, with concentrations up to 3.2 mg/kg. Only 17% was excreted via urine (Hedman *et al.*, 1997a).

4.2.1.2 Metabolism

When incubated for 48 h with cow ruminal fluid, 80% of NIV was de-epoxidated. The same result was obtained for DON (Hedman and Pettersson, 1997).

After repeated oral administration, the main metabolite of NIV in the excreta of male rats was de-epoxy-NIV (80% and 1% of the total dose in faeces and urine, respectively). The parent compound NIV was detected at much lower levels (7% and 1% of the total dose in faeces and

urine, respectively). Gastrointestinal microorganisms likely participate in the de-epoxidation (Onji *et al.*, 1989). After single oral dosing de-epoxy-NIV was excreted predominantly in faeces (18.7% vs. 5.6% in urine), and the excretion was about 24 h later than that of NIV (9.9% in urine and 5.4% in faeces) (Onji, 1990).

When male swine were fed for up to one week with NIV in the diet at a concentration of 2.5 mg/kg (corresponding to a dose of 0.1 mg/kg bw/day), no metabolites of NIV could be found in plasma, urine or faeces, either as glucuronic acid or sulphate conjugates, or as de-epoxy-NIV, indicating a lack of metabolism (Hedman *et al.*, 1997a). However, when male swine were fed diets containing 2.5 or 5 mg/kg NIV for up to three weeks, from one week of exposure onwards, over 90% of NIV in faeces was in the de-epoxy-NIV form. Also in bile 32-44% of NIV was in the de-epoxy form. Apparently, it takes a few days for the gastrointestinal microflora to adapt itself to form de-epoxide metabolites. Once adapted, these microorganisms were also able to produce de-epoxidated metabolites of DON *in vitro* (Hedman and Pettersson, 1997; Pettersson and Hedman, 1997).

In contrast to rats and pigs, in faeces of male broiler chickens fed NIV in the diet at 2.5 or 5 mg/kg for three weeks, no de-epoxy-NIV was found. An unknown metabolite was found, though, in varying amounts. Presumably this is an acetylated metabolite of NIV (Hedman and Pettersson, 1997).

4.2.2 Toxic effects

4.2.2.1 Acute toxicity

In 6-week old male ddY mice NIV appeared to be less toxic after oral administration (LD_{50} of 38.9 mg/kg bw) than after intraperitoneal (ip), intravenous (iv) or subcutaneous (sc) administration (LD_{50}-values of 7.4, 7.3 and 7.2 mg/kg bw, respectively). Independent of route, most deaths occurred within three days after administration. Histopathology revealed a marked congestion and haemorrhage in the intestines (Ryu *et al.*, 1988). When given ip or sc to male Swiss mice, the LD_{50}-values for NIV were 9.6 and 7.3 mg/kg bw, respectively (Thompson and Wannemacher, 1986). Ueno (1984) reported LD_{50}-values in 6-week old male ddyS mice of 4.1 and 6.3 mg/kg bw after ip and iv administration, respectively. Postmortem examination revealed extensive haemorrhages in the intestine and diarrhoea, and cellular destruction in the epithelial mucous membranes of intestine, in the thymus and testis. An identical ip LD_{50} of 4.1 mg/kg bw in male mice was found by Ueno *et al.* (1973a), with NIV inducing radiomimetic cellular injury and karyorrhexis in (amongst others) the small intestine and bone marrow.

For male and female F344 rats, an oral LD_{50} of 19.5 mg/kg bw has been reported for NIV by Kawasaki *et al.* (1990). Effects observed were sedation, eyelid closure, staggering gate, diarrhoea and congestion of the lungs and digestive tract. Ueno (1983a) reported an LD_{50} of 0.9 mg/kg bw after sc administration of NIV to rats.

4.2.2.2 Subacute toxicity

When administered NIV in the diet at concentrations of 0, 5, 10 or 30 mg/kg for 24 days, feed utilization efficiency and body weight gain of female C57BL/6CrSlc spf mice were dose-relatedly (but not statistically significantly) reduced at 10 and 30 mg/kg. At 30 mg/kg, mice developed significant erythrocytopenia and slight leukopenia, and upon ultrastructural examination bone marrow cells revealed polyribosomal breakdown. No marked changes were observed on haemoglobin and haemotocrit and on weights of the liver, spleen and thymus. No histopathological changes were found in thymus, spleen, liver, stomach, ovary, uterus, lymph node and small intestine with or without Peyer's patches (Ryu *et al.*, 1987).

Oral gavage administration of 0, 0.4 or 2 mg NIV/kg bw to male and female F344 rats for 15 or 30 days did not affect haematological or biochemical parameters. At 2 mg/kg bw the liver and spleen weights were significantly increased, but no histopathological changes were seen (Kawasaki *et al.*, 1990).

Groups of 5 male SD rats were administered 0, 6, 12 or 30 mg NIV/kg diet for 2-4 weeks. The highest dose caused mortality within 6-8 days due to marked feed refusal and reduced body weight gain. Reductions in feed consumption and body weight gain were also observed at 6 and 12 mg/kg, but only in the first 1-2 weeks. After 2 weeks of treatment, absolute and relative weights of liver and spleen were decreased at 12 mg/kg, while kidney weights were not affected. In contrast, after 4 weeks of treatment, absolute and relative weights of liver and kidney were increased at 12 mg/kg and even more so at 6 mg/kg, while spleen weights were still decreased at 12 mg/kg but less than after 2 weeks. Among the serum parameters investigated, asparate aminotransferase was dose-relatedly decreased (statistically significantly only at 12 mg/kg) (Yabe *et al.*, 1993). Effects on hepatic drug-metabolizing acitivity and aflatoxin B1 metabolism are described in 4.2.2.8.

Administration of 0, 10 or 50 mg NIV/kg diet to male rats for 8 weeks only affected the high dose animals. They showed decreased body weight gain, weak erythrocytopenia, mucosal necrosis and disruption of the gut epithelium, and an increase of erythroid series in bone marrow (Onji, 1990).

The feeding of NIV at a dose of 0.05 mg/kg bw, twice daily for 20 days, did not cause feed refusal in male swine nor changes in clinical plasma parameters (Hedman *et al.*, 1997a). When groups of 6 male swine were fed with 2.5 or 5 mg NIV/kg diet for up to three weeks (corresponding to doses of 0.1 and 0.2 mg NIV/kg bw/day), no effects were observed on behaviour or appearance, on body weight or weight gain, on food consumption or on the weights of liver, kidney, thymus, spleen and heart. No swine vomited. However, in contrast to control swine, swine of both treated groups showed mild pathological changes in the gastrointestinal tract (changes in thickening of mucosa and haemorrhagic lesions), kidneys (pale, with narrow cortex and dilated renal pelvis, and cysts) and spleen (apex infarcts). Between control and treated swine there were no differences in total or differential blood

leucocyte counts, nor in the number of thymocytes or in plasma cortisol levels. Treatment with NIV, however, induced a dose-dependent decrease in spleen cells (statistically significant only at 5 mg/kg), which was reflected in decreased numbers (but not proportions) of CD4+, CD8+ and IgM+ subpopulations in the spleen (Hedman *et al.*, 1997b). Effects on plasma immunoglobulin levels and on immune function *in vitro* are described in 4.2.2.7.

Dietary treatment of 7-day old male broiler chickens with 0.5 – 12 mg NIV/kg for 20 days resulted in decreased body weight gain (by 11%) at 6 and 12 mg/kg. At these dosages feed consumption was also decreased (by 6-7%), while feed conversion efficiency was increased (by approximately 5%). Gizzard erosions were found in 33% of the birds fed 12 mg/kg and in 8% of those fed 3 or 6 mg/kg, but not in controls. Absolute and relative liver weights were decreased at 6 and 12 mg/kg. Although no effects were found on relative weights of bursa, spleen and gizzard, for the latter the absolute weight was decreased at 3 and 12 mg/kg. In blood, no change compared to control was found in haematocrit or in the plasma concentration of glucose, calcium, cholesterol, triglycerides and uric acid, or in the plasma activity of aspartate amino transferase, alanine amino transferase or gamma glutamyl transpeptidase. Histopathology of liver, thymus and spleen revealed no treatment-related changes (Hedman *et al.*, 1995).

4.2.2.3 Subchronic toxicity

Groups of 20 male and 20 female C57BL/6CrSlc spf mice were given diets containing 0, 6, 12 or 30 mg/kg NIV for 12 weeks, with an interim kill of 10 animals/sex/group after 4 weeks. Observations included body weight and feed consumption, serum biochemistry, organ weights (liver, thymus, spleen and kidneys) and histology (liver, thymus, spleen, kidneys, stomach, adrenal glands, pituitary gland, ovaries, sternum, femurs, mesenterial lymph node, brain and small intestines with or without Peyer's patches).
NIV treatment caused a dose-related reduction in body weight gain in both males and females: after 12 weeks the weight gain at 6, 12 and 30 mg/kg, as compared to that of controls, was 98, 77 and 30% for males and 70, 55 and 45% for females, respectively. Feed consumption was also reduced, and in particular the high dose animals showed slight feed refusal in the first week. Absolute and relative weights of liver, kidney, spleen and thymus were variably affected by NIV treatment, without clear trends. No gross or histopathological changes were seen in the tissues and organs examined but treated groups had considerably less fatty tissue at autopsy than controls. Among the serum parameters examined, only one was consistently affected: in both males and females the alkaline phosphatase activity was dose-relatedly increased at all doses after 4 and after 12 weeks (Yamamura *et al.*, 1989).

Groups of 12 female C57BL/6CrSlc spf mice were given diets containing 0, 6, 12 or 30 mg/kg NIV for 1 year (equal to 0, 0.68-0.76, 1.51-1.64 or 3.84-3.95 mg NIV/kg bw/day), with an interim kill of 6 animals/group after 6 months. Observations included body weight and feed consumption, haematology, organ weights (liver, thymus, spleen and kidneys), histology (liver, thymus, spleen, kidneys, stomach, adrenal glands, pituitary gland, ovaries,

sternum, bone marrow, lymph node, brain and small intestines with or without Peyer's patches) and ultrastructural studies of the bone marrow.
Body weight gain was dose-relatedly reduced (after 1 year, the weight gain at 6, 12 and 30 mg/kg was 79, 69 and 40% of that of controls, respectively). Feed efficiency was also dose-relatedly reduced. Feed refusal was only seen at 12 and 30 mg/kg in the first 3-5 weeks. Feed consumption was reduced at all doses, but only marginally (by <10%) at 6 and 12 mg/kg. Both after 6 months and after 1 year of treatment, absolute weights of liver, kidney and thymus were dose-dependently decreased (statistically significant at 30 mg/kg), while relative weights of these organs plus spleen were dose-dependently increased (statistically significant at 12 and/or 30 mg/kg). The organ weight changes were not accompanied by histopathological changes. NIV caused a severe leukopenia, which after 6 months was only observed at 30 mg/kg but after 1 year at all doses. Other haematological parameters were not affected. In contrast to the findings in the subacute feeding study (Ryu *et al.*, 1987), no erythrocytopenia and no damage to the bone marrow were observed (Ryu *et al.*, 1988).

4.2.2.4 Chronic toxicity/Carcinogenicity

Groups of 42 female C57BL/6CrSlc spf mice were given diets containing 0, 6, 12 or 30 mg/kg NIV for 2 years (equal to 0, 0.66, 1.38 or 3.49 mg NIV/kg bw/day). Observations included body weight and feed consumption, clinical signs and mortality, haematology and serum biochemistry at autopsy, organ weights (liver, thymus, spleen, kidneys and brain) and histology (on all tumours and on liver, thymus, spleen, kidneys and brain).
During the dosing period body weight gain and feed efficiency were dose-relatedly reduced. However, due to the earlier death of the control and low dose animals (see below), the body weight changes were not apparent from the terminal weights. Initial feed refusal was seen at 12 and 30 mg/kg. Feed consumption was reduced at all doses, but only marginally (by <10%) at 6 and 12 mg/kg. No significant changes in relative organ weights were observed. Significant decreases in absolute organ weight were found for liver (at 30 mg/kg) and kidney (at 12 and 30 mg/kg). In contrast to the 1-yr study (Ryu *et al.*, 1988), only slight leukopenia accompanied by a slight decrease in lymphocytes was seen (at 12 and 30 mg/kg, but not at 6 mg/kg). Dose-related increases in alkaline phosphatase activity, aspartate aminotransferase and non-esterified fatty acids, and decreases in alanine aminotransferase, amylase, creatinine phosphokinase and calcium were observed, but the changes were statistically significant only at 30 mg/kg. Compared to controls, NIV treatment increased the lifespan. Except for amyloidosis (especially in the small intestine) and tumour development (especially lymphomas) no histopathological changes were observed. Controls and low dose animals had the highest incidence of amyloidosis, and this was a major cause of (early) death in these groups. Tumour development was not related to NIV treatment. Tumours occurred in similar incidences in control and treated groups, but appeared to develop later and grow slower at 30 mg/kg. The low mortality rate at 30 mg/kg might be due to the lower incidence of amyloidosis and the lower tumour incidence in the earlier part of the study (Ohtsubo *et al.*, 1989). This study was also evaluated by IARC (1993), and they noted the limited number of tissues studied.

One-week old C57BL/6xC3H F1 mice received a single ip injection with 6 mg/kg bw aflatoxin B1 (AFB1), 6 weeks later followed by dietary treatment with 0, 6 or 12 mg/kg NIV for 1 year. The mice were killed at 71 weeks of age. All male mice treated with AFB1 developed liver tumours (mainly carcinomas), and feeding with NIV did not affect the incidence. The incidence of liver tumours and their degree of malignancy was much lower in female mice treated with AFB1, and NIV treatment had a tumour-suppressing effect on AFB1-induced hepatocarcinogenesis, presumably by acting on the promotion step (Ueno *et al.*, 1991).

In an *in vivo* medium-term bioassay using glutathione S-transferase placental form (GST-P)-positive liver foci as endpoint in six-week old male F344 rats initiated by diethylnitrosamine (DEN), Ueno *et al.* (1992) investigated whether NIV possessed hepatocarcinogenic potential, and secondly, whether AFB1-induced hepatocarcinogenicity was modulated by exposure to NIV. In the first experiment, treatment with AFB1 (1x ip 0.5 mg/kg bw) resulted in a small formation of GST-P-positive liver foci in non-DEN-initiated rats, and this induction was accelerated in DEN-initiated rats (1x ip 200 mg/kg bw), confirming the hepatocarcinogenicity of AFB1. No induction of GST-P-positive liver cell foci by NIV (6 mg/kg diet for 6 weeks) was observed in rats either or not initiated with DEN, hence predicting no hepatocarcinogenic potential for NIV. However, feeding of 6 mg/kg NIV for 6 weeks to DEN-initiated and AFB1-treated rats synergistically enhanced the formation of GST-P-positive liver cell foci (not in non-DEN initiated and AFB1-treated rats). Hence, NIV is predicted to cause an enhancing effect on AFB1-induced hepatocarcinogenesis.

4.2.2.5 Genotoxicity

When studied for chromosomal damage, induction of sister chromatid exchanges and cell cycle delay in Chinese hamster V79-E cells *in vitro*, NIV showed only weak activity, more so with metabolic activation than without. The marginal effects observed are probably unspecific and are caused by inhibition of protein synthesis (Thust *et al.*, 1983). An induction of chromosomal aberrations in Chinese hamster V79 cells *in vitro* was also observed by Hsia *et al.* (1988) when testing NIV (both pure and extracted from corn), but not by Ryu *et al.* (1993).

In the alkaline single-cell gel electrophoresis (or COMET) assay, NIV was tested for DNA damage *in vitro* in Chinese hamster ovary cells (without metabolic activation) and *in vivo* in male ICR mice after oral and ip administration (using seven organs: stomach, jejunum, colon, liver, kidney, thymus and bone marrow). NIV was positive *in vitro*. *In vivo*, DNA damage was seen in colon (both after ip and oral treatment), in kidney, bone marrow, stomach and jejunum (after oral treatment only), but not in liver and thymus. NIV showed organ specific genotoxicity in time and intensity: in the colon DNA damage was strong yet delayed, while DNA damage in stomach, kidney and bone marrow was strong at the onset but decreased with time. The *in vivo* findings were not secondary to cytotoxicity, as no apoptotic cells and

no histopathological findings including necrotic changes were detected in any organ (Tsuda *et al.*, 1998).

4.2.2.6 Reproductive and Developmental toxicity

In a study by Ito *et al.* (1986) pure NIV was injected ip in pregnant ICR mice at dose levels of 0, 0.1, 0.5 or 1.5 mg/kg bw/day on days 7-15 of gestation. The highest dose caused stillbirths after vaginal haemorrhage in 6 out of 10 animals. High embryolethality was recorded in the two highest dose groups (88 and 48%). No fetal malformations were observed in the treated groups. A single administration of 3 mg/kg bw on day 7 affected the embryo within 10 h, damaged the placenta within 24 h, and caused stillbirths at 48 h.

Mice were fed diets with mouldy rice powder containing NIV at final levels of 0, 6, 12 or 30 mg/kg feed throughout gestation. Additional mice were administered purified NIV by gavage at doses of 0, 1, 5, 10 or 20 mg/kg bw on days 7-15 of gestation. Four of five mice given 20 mg/kg bw died during the dosing period. Embryotoxicity associated with maternal weight loss was observed in the groups receiving 30 mg/kg and 10 mg/kg bw. Intrauterine growth retardation was found in these groups, and also in the term fetuses of mice exposed to 12 mg/kg and 5 mg/kg bw. NIV had no statistically significant adverse effects on the incidence of gross, skeletal and visceral malformations (Ito *et al.*, 1988).

4.2.2.7 Immunotoxicity

NIV given in the diet at concentrations of 0, 6 or 12 mg/kg for 4 or 8 weeks reproducibly induced some pathological changes in mice which resemble those in human IgA nephropathy: irrespective of mice strain (C3H/HeN, C3H/HeJ or BALB/c), NIV induced IgA deposits in the glomerular mesangium and elevated IgA levels, and the degree of these changes was associated with the dose and duration. As it is known that IgA production in the body is primarily by the mucosal immune system, it is hypothesized that NIV reaches the gut of mice to dysregulate their mucosal immune system resulting in the pathogenesis of IgAN (Hinoshita *et al.*, 1997).

Depending on dose and time, NIV and other trichothecenes like T-2, DON, 3- and 15-Ac-DON, could inhibit or superinduce cytokine production and mRNA expression in murine spleen CD4+ T cells. The rank order of superinduction was T-2 > DON, NIV > 15-Ac-DON > 3-Ac-DON (Ouyang *et al.*, 1995).

Dietary treatment of male swine with 2.5 or 5 mg NIV/kg diet for up to three weeks (corresponding to doses of 0.1 and 0.2 mg NIV/kg bw/day) did not affect IgA and IgG plasma levels at any of the samplings (after 0, 1 and 3 weeks of treatment). The *in vitro* production of both IgG and IgA by mitogen-stimulated lymphocytes was not affected by NIV, and neither was the mitogen-induced proliferation of lymphocytes from blood, spleen or thymus. Apparently, the functioning of the immune system was not affected by NIV. In the 2.5 mg/kg feed group there were some time-dependent changes from the first to the third

week of exposure (decrease in plasma IgG and increase in IgA levels; the levels of IgA and IgG in plasma, though, did not significantly differ from controls at these samplings (see above)). NIV at 2.5 mg/kg feed also decreased the *in vitro* production of IgG by mitogen-stimulated splenocytes but not the *in vitro* production of IgA. However, the effects at 2.5 mg/kg feed were not observed in the 5 mg/kg feed group (Hedman *et al.*, 1997b).

NIV inhibited blastogenesis in cultured human lymphocytes. It produced a 50% inhibition of [^{3}H]thymidine uptake in mitogen-stimulated human lymphocytes at a concentration of 72 ng/ml. Other trichothecenes like T-2, HT-2, FusX, DON and 15-Ac-DON produced 50% inhibition in this system at concentrations of 1.5, 3.5, 18, 140 and 240 ng/ml, respectively. These results indicate that the lymphotoxicity of trichothecenes is related to the C4 substituent: acetyl>hydroxyl>hydrogen. Acetylation at C15 slightly decreases lymphotoxicity (Forsell and Pestka, 1985; Forsell *et al.*, 1985).

Thuvander *et al.* (1999) found that NIV effectively inhibited proliferation and immunoglobulin production (IgA, IgG and IgM) in mitogen-stimulated human lymphocytes in a dose-dependent manner with limited variation in sensitivity between individuals. However, at low levels NIV exposure could also result in enhanced proliferative responses, as well as in elevated immunoglobulin production (especially IgA). NIV was about as effective as DON, but less effective than T-2 (100-250 times more potent) and DAS (25-80 times more potent). Combinations of NIV with T-2, DAS or DON resulted in additive toxicity.

In an *in vitro* test with human peripheral blood mononuclear cells, NIV significantly and dose-dependently inhibited the T and B cell proliferation. This effect was also observed for T-2, FusX, DON and 3-Ac-DON, but hardly or not at all for α- and β-zearalenol. NIV also suppressed natural killer cell activity (dose-dependent) and inhibited antibody-dependent cellular cytotoxicity reaction. This was also the case for T-2 and DON, but was not investigated for the other mycotoxins (Berek *et al.*, 2001).

When investigated in ovalbumin-specific T cell receptor αβ-transgenic mice (i.e. mice which respond with sensitivity to oral administration of ovalbumin as a dietary constituent by exhibiting an increase in serum IgE, the causative immunogloblin of food allergy), NIV inhibited antigen-induced total and antigen-specific IgE production in association with inhibition of IL-4 production and enhancement of IL-2 production (Choi *et al.*, 2000).

4.2.2.8 Other effects

For NIV, the lowest sc dose causing vomiting in ducklings was 1 mg/kg bw (Ueno, 1983a).

When applied to the shaved back skin of guinea pigs, NIV induced redness 2-10 hrs after painting (effective dose 10^{-8} mol). Compared to T-2, HT-2, DAS and type D trichothecenes (effective doses 10^{-10} - 10^{-11} mol), NIV was low in dermal toxicity (Ueno, 1984).

As can be seen from Table 8 in 4.1.2.2, NIV is cytotoxic to cultured cells and possesses protein synthesis inhibiting activity. In HeLa cells, NIV inhibited multiplication at every phase of the growth cycle. In a variety of systems (amongst which Ehrlich ascites tumour cells and HeLa cells), NIV has been found to inhibit not only protein but also DNA synthesis, while RNA synthesis was marginally affected (Saito and Ohtsubo, 1974).

After infection of human epidermoid carcinoma no.2 (HEp-2) cells with herpes simplex virus types 1 and 2, NIV exerted antiviral activity by inhibiting plaque formation (EC_{50}s of 120 and 50 ng/ml, respectively). NIV affected virus replication after but not before or during virus adsorption to the host cells. The antiviral activity of NIV was less than that of FusX (EC_{50}s of 56 and 26 ng/ml, respectively) but greater than that of DON (EC_{50}s of 160 and 94 ng/ml, respectively), suggesting that the potency is related to the C4 substituent: acetyl>hydroxyl>hydrogen. Possibly the inhibitory effect can be brought about by binding of the trichothecenes to the polyribosomes in the viral protein synthesis (Tani *et al.*, 1995).

When given at 6 or 12 mg/kg feed for 2-4 weeks to rats, NIV induced the activities of microsomal cytochrome P-450 (isozymes 2B1/2 and 1A2) and cytochrome b5, drug-metabolizing enzymes (aminopyrine-*N*-demethylase and aniline hydroxylase) and cytosolic glutathione S-transferase (isozyme 1-2). The induction of microsomal cytochrome P-4502B1/2 and the increase in microsomal drug metabolism was transient, as the levels at 4 weeks were less than those at 2 weeks. This ability of NIV to induce both phase I and phase II enzyme systems for drug metabolism resulted in a modulation of AFB1 adduction to DNA *in vitro* and *in vivo. In vitro*, the formation of AFB1-DNA adducts was increased using hepatic microsomal preparations from rats fed NIV, whereas supplementation with cytosol prepared from NIV-treated rats reduced the microsomal potential for adduct formation. *In vivo*, the hepatic AFB1-DNA concentration was lower in NIV-treated rats than in controls (Yabe *et al.*, 1993).

4.3 Fusarenon X (FusX)

4.3.1 Toxicokinetics

4.3.1.1 Absorption, distribution and excretion

Thirty minutes after sc administration of uniformly labelled ^{3}H-FusX at 4 mg/kg bw to mice, activity was found in liver, kidneys, intestines, stomach, spleen, bile and plasma; none was detected in heart, brain or testis. The highest activity was found in the liver (3% of the dose). Twelve hrs after administration, no label was present in the organs, and 25% of the dose was recovered as metabolised forms of FusX in the urine (Ueno *et al.*, 1971).

4.3.1.2 Metabolism

FusX is deacetylated to NIV by rat and rabbit liver carboxy esterases (Ohta *et al.*, 1978).

4.3.2 Toxic effects

4.3.2.1 Acute toxicity

When given ip or sc to male Swiss mice, the LD_{50}-values were 5.6 and 5.5 mg/kg bw, respectively (Thompson and Wannemacher, 1986). A slightly lower ip LD_{50} of 3.3 mg/kg bw in male mice was found by Ueno *et al.* (1973a), with FusX inducing radiomimetic cellular injury and karyorrhexis in (amongst others) the small intestine and bone marrow. Ueno (1984) reported LD_{50}-values in 6-week old male ddyS mice of 4.5, 3.4, 4.2 and 3.4 mg/kg bw after oral, ip, sc and iv administration, respectively. Newborn mice, with an sc LD_{50} of 0.23 mg/kg bw, were more susceptible than 6-week old mice. Postmortem examination revealed extensive haemorrhages in the intestine and diarrhoea, and cellular destruction in the epithelial mucous membranes of intestine, in the thymus and testis.
FusX was also tested for acute toxicity in rats (LD_{50}-values of 0.5 and 4.4 mg/kg bw for sc and oral administration, respectively), guinea pigs (LD_{50}-values of 0.5 and 0.1 mg/kg bw for ip and sc administration, respectively), cats (sc LD_{50} <5 mg/kg bw) and ducklings (sc LD_{50} 2 mg/kg bw) (Ueno, 1983a).

Early after injection of FusX in mice, a rapid increase of leucocytes and lymphocytes was seen, accompanied by increased β-globulin and decreased γ-globulin. On autopsy, cellular degeneration and karyorrhexis were marked in bone marrow and small intestine (Ueno et al., 1973b).

4.3.2.2 (Sub)Chronic toxicity/Carcinogenicity

Saito and Ohtsubo (1974) reported several experiments with repeated oral and sc administration of FusX to male Donryu rats and male DDD mice. The description of these experiments is rather limited, which was also noted by IARC (1993).
In the first experiment, 20 rats were administered weekly 0.4 mg FusX/kg bw by oral intubation for 50 weeks. Of the 12 rats that survived 50 weeks, one developed a hepatoma. Histopathology revealed intrahepatic bile duct hyperplasia and atypical hyperplasia in the gastric and intestinal mucosa in several rats. Hypoplasia and atrophy of the bone marrow, thymus and spleen were observed in about half of the animals.
When given the same dose weekly by sc injection for 22 weeks, most of the 18 rats survived more than 1 year. Similar histopathological lesions were observed. There was one animal with lung adenoma.
Two groups of 16 or 18 mice received 10 or 20 weekly sc injections of 2.5 mg FusX/kg bw. Most of the animals survived the treatment. Local alopecia at the injection site was seen but the hair regenerated in a few months. Pathological changes were scarce except for moderate thymus atrophy. One case of leukaemia was observed.

It was stated that in control animals (10 rats and 11 mice) no tumour occurred during the experimental period of over 400 days, and that atrophy of the organs was mild and observed in a few cases only.

Male Donryu rats were given diets containing 3.5 (n=49) or 7 (n=25) mg FusX/kg for two years. Two additional groups of 26 animals were given the highest dose for one year followed by control diet for one year, with one group also receiving 20 oral administrations of penicillic acid (50 mg/animal) during the first year. There were 48 controls receiving no treatment, and 25 controls receiving 20x penicillic acid. All animals were given a restricted volume of feed (15 g/day), to provide 50 and 105 µg FusX per day per animal. Survivors were killed at 24 months.
The mean body weights of the treated animals were in general lower than those of the control animals. Body weight gain recovered after withdrawal of the experimental diet. Survival was poor and treatment-relatedly affected: after 18 months of treatment, 50% (24/48) of the controls were alive, compared with 15/49 (31%) and 4/25 (16%) in the low and high dose group, respectively. Survival at that time in the groups receiving the high dose for one year was 9/52 (17%), while that for the penicillic acid controls was 9/25 (36%). After 24 months the number of surviving animals was 15/48 and 7/25 for controls and 5/49, 0/25 and 6/52 for treated groups. The major cause of death in both control and treated animals was chronic bronchopneumonia, but the incidence was higher in treated animals. No increase in the incidence of tumours was observed in treated rats (Saito *et al.*, 1980). This study was also evaluated by IARC (1993), and they noted the poor survival of the treated animals.

In a skin painting study, groups of 10 female ICR mice each received topical applications (on the back) of 2 or 20 µg FusX twice a week for 25 weeks. Other groups of mice received topical applications of 51.2 µg 7,8-dimethylbenz(a)anthracene (DMBA), two weeks later followed by 2 or 20 µg FusX twice a week for 23 weeks. Groups of 10 positive control mice received twice weekly a topical application of 10.5 µg phorbol-12-myristate-13-acetate (TPA), with or without DMBA pretreatment. Eight of 10 mice receiving DMBA plus TPA developed skin papillomas on their backs 4-8 weeks after the start of treatment. No skin papilloma or tumorous changes were found in mice receiving 2 or 20 µg FusX alone or 20 µg FusX in combination with DMBA. One mouse treated with DMBA plus 2 µg FusX developed several small skin papillomas after 21 weeks (Ueno, 1984).

4.3.2.3 Genotoxicity

When tested in Chinese hamster V79-E cells *in vitro*, FusX provoked marked toxicity as evidenced by cell cycle delay. Compared to toxicity, FusX was only weakly clastogenic, by inducing chromosomal aberrations and sister chromatid exchanges. Metabolic activation had no influence on FusX activity. The marginal effects observed are probably unspecific and are caused by inhibition of protein synthesis (Thust *et al.*, 1983).

Besides the weak clastogenic response in Chinese hamster cells *in vitro*, IARC (1993) reported that FusX did not induce DNA damage in the Rec-assay using *Bacillus subtilis*, was not mutagenic to *Salmonella typhimurium* and did not induce gene mutations in cultured mouse mammary carcinoma FM3A cells. However, at high doses FusX increased the frequency of petite mutations in yeast and (weakly) induced DNA single-strand breaks in cultured human HeLa cells. For the latter, however, a negative result was reported by WHO (1990).

4.3.2.4 Reproductive and Developmental toxicity

Groups of pregnant female DDD mice received FusX by sc injection either at single doses of 0.63, 1, 1.6, 2.6 or 4.1 mg/kg bw on day 10 of gestation, or at a single dose of 1.6 mg/kg bw on day 6, 8 or 13 of gestation, or at multiple doses of 0.63, 1 or 1.6 mg/kg bw on days 8-12 or 8-14 of gestation. While the dams given 4.1 mg/kg bw died within 24 h of injection, all dams receiving 2.6 mg/kg bw aborted one day after injection. With lower doses of 1.6, 1 and 0.63 mg/kg bw, abortion occurred less frequently (16-20%) with longer intervals after injection (2-7 days), but embryotoxicity as evidenced by a dose-dependent increase in the number of resorbed and dead fetuses (22%, 32% and 40% at 0.63, 1 and 1.6 mg/kg bw, respectively) was seen. When compared to a single dose of 1.6 mg/kg bw on day 10 of gestation, the same dose induced more abortions when given on day 6 (75%) or 13 (50%) but none when given on day 8. The later the day of injection with 1.6 mg/kg bw, the more fetal absorptions occurred. Multiple doses of 1.6 and 1 mg/kg bw caused all animals to abort, while no animal aborted at multiple doses of 0.63 mg/kg bw. Compared with control fetuses, body weight and body length of the surviving fetuses were reduced, especially when given a single dose of 1.6 mg/kg bw on day 6 or 8 of gestation, or multiple doses of 0.63 mg/kg bw. No teratogenic effects were seen (Ito *et al.*, 1980).

In the same experiment, pregnant female DDD mice were fed diets mixed with FusX at concentrations of 5, 10 or 20 mg/kg diet (approximately 25, 50 or 100 µg FusX/animal/day) throughout gestation or during early, mid or late pregnancy. When given throughout gestation or during the early stages of gestation, FusX inhibited embryonal implantation and induced abortion (in 60-100% of the animals), fetal absorption and fetal growth retardation (weight). When given during mid gestation (day 7-14), Fus X induced abortion (dose-and duration-dependent) and fetal absorption, as well as growth retardation (weight and length) in surviving fetuses. The latter was also found when mice were fed FusX during late gestation. Teratogenic effects were not apparent (Ito *et al.*, 1980).

4.3.2.5 Immunotoxicity

Repeated daily ip administration of FusX resulted in an immunosuppressive effect in BALB/c mice. IgE and IgG1 antibody formation *in vivo*, as well as *in vitro* antibody formation by splenic lymphocytes raised by T-dependent and independent mitogens, was suppressed (Masuda *et al.*, 1982; IARC, 1993).

FusX inhibited blastogenesis in cultured human lymphocytes. It produced a 50% inhibition of [^{3}H]thymidine uptake in mitogen-stimulated human lymphocytes at a concentration of 18 ng/ml. Other trichothecenes like T-2, HT-2, NIV, DON and 15-Ac-DON produced 50% inhibition in this system at concentrations of 1.5, 3.5, 72, 140 and 240 ng/ml, respectively. These results indicate that the lymphotoxicity of trichothecenes is related to the C4 substituent: acetyl>hydroxyl>hydrogen. Acetylation at C15 slightly decreases lymphotoxicity (Forsell and Pestka, 1985; Forsell *et al.*, 1985).

In an *in vitro* test with human peripheral blood mononuclear cells, FusX significantly and dose-dependently inhibited the T and B cell proliferation. This effect was also observed for T-2, NIV, DON and 3-Ac-DON, but hardly or not at all for α- and β-zearalenol. For T-2, NIV and DON, the immunomodulatory effect on mononuclear cells also included a suppression in natural killer cell activity and an inhibition in antibody-dependent cellular cytotoxicity reaction, but this was not investigated for FusX (Berek *et al.*, 2001).

4.3.2.6 Other effects

FusX induced hypothermia but did not produce significant behavioural changes in mice. In anaesthetized rats, FusX caused a rise in blood pressure and a decrease in respiratory rate but induced no significant change in heart beat rate. In isolated tissues such as fundus muscle, vas deferens, tracheal muscle, ileum and atrium, no detectable effects on spontaneous activity and responsiveness to agonists were observed in the presence of FusX, indicating that no attack on the muscle cell membrane or the nerve elements of the tissues takes place (Ueno, 1983a).

In rats, ip injection of FusX caused watery diarrhoea 36-60 h after injection, and expansion of the small intestine was marked 24 h after injection. Histological and functional damage to the intestinal mucosa was observed. The impairment of intestinal permeability is presumed to be related to trichothecene-induced diarrhoea (Ueno, 1983a).

For FusX, the lowest sc dose that caused vomiting in ducklings and cats was 0.4 and 1 mg/kg bw, respectively. When tested in dogs the lowest effective dose after iv administration was 0.1 mg/kg bw. Studies with FusX in dogs revealed that the vomiting action is possibly due to a trichothecene-induced stimulation of the chemoreceptor trigger zone in the medulla oblongata (Ueno, 1983a).

When applied to the shaved back skin of guinea pigs, FusX induced redness 2-10 hrs after painting (effective dose 10^{-9} mol). FusX was of intermediate dermal toxicity when compared to T-2, HT-2, DAS and type D trichothecenes (effective doses 10^{-10} - 10^{-11} mol) and NIV, 3-Ac-DON and DON (effective doses 10^{-7} – 10^{-8} mol). Studies with FusX into the mechanism of dermal toxicity suggest that trichothecenes directly attack the capillary vessel and increase its permeability (Ueno, 1984).

As can be seen from Table 8 in 4.1.2.2, FusX is highly cytotoxic to cultured cells and is a potent inhibitor of protein synthesis. In a variety of systems (amongst which Ehrlich ascites tumour cells and HeLa cells), FusX has been found to inhibit not only protein but also DNA synthesis, while RNA synthesis was not or only marginally affected (IARC, 1993).

After infection of human epidermoid carcinoma no.2 (HEp-2) cells with herpes simplex virus types 1 and 2, FusX exerted antiviral activity by inhibiting plaque formation (EC_{50}s of 56 and 26 ng/ml, respectively). FusX affected virus replication after but not before or during virus adsorption to the host cells. The antiviral activity of FusX was greater than that of NIV (EC_{50}s of 120 and 50 ng/ml, respectively) and DON (EC_{50}s of 160 and 94 ng/ml, respectively), suggesting that the potency is related to the C4 substituent: acetyl>hydroxyl>hydrogen. Possibly the inhibitory effect can be brought about by binding of the trichothecenes to the polyribosomes in the viral protein synthesis (Tani *et al.*, 1995).

4.4 Diacetoxyscirpenol (DAS)

4.4.1 Toxicokinetics

4.4.1.1 Absorption, distribution and excretion

Tissue distribution and excretion in male Fischer rats and CD-1 mice were examined at 90 min, 24 hrs and 7 days following a single intragastric dose of [^{3}H]DAS proportional to body weight (approximately 0.66 mg/kg bw for mice and 0.55 mg/kg bw for rats). These doses did not elicit visible signs of toxicity or damage to tissues. In both species, urinary and faecal excretion were very rapid and essentially completed within the first 24 hrs, with approximately 90% of the dose excreted. The ratio of urinary to faecal excretion was approximately 4.5:1 for both rat and mouse. The rapid excretion in the first 24 hrs was in both species paralleled by a concomitant rapid decline of radiolabel in gastrointestinal tract contents, organs and carcass, in which the radioactivity plateaued at low but significant levels (1.4-2.8% in rats, 2.8-3.4% in mice) over the remaining six days. When expressed as percentage of dose, tissue distribution in the rat and mouse was quantitatively similar, with most radiolabel in carcass, skin, small intestine, stomach, liver and kidney. The rank order was generally stable over the course of the experiment. When expressed as specific radioactivity, carcass and skin became less important and were replaced by spleen and cecum. With time, the rank order shifted to include the lympho-haematopoietic system (spleen, thymus and femur bone marrow), heart and (in mouse) testis, as well as cecum and large intestine. In these target organs the decrease in radioactivity with time was less than for liver, kidney, skin and carcass. Radioactivity in the brain, although low, diminished relatively slowly.

The very few interspecies differences tended to occur in target organs (with the higher levels present in the mouse), and in small intestine (higher levels for rats) and kidney and liver

(higher levels for mice). These latter findings suggest that the mouse is somewhat more efficient than the rat in metabolizing and excreting DAS (Wang *et al.*, 1990).

The same authors repeated the above mentioned experiment, but now with a single topical application of [^{3}H]DAS proportional to body weight (0.98 mg to 1.44 cm^2 of skin for rats, 0.28 mg to 0.42 cm^2 of skin for mice) (Wang *et al.* (1996)). After topical administration, rats and mice exhibited different patterns of absorption, excretion and tissue distribution over the time period of 90 min to 7 days after application. In the rat, absorption and excretion increased during this time period, while retention in tissues decreased. In mice, however, absorption from the application site occurred only during the first 24 hrs (also reflected in the amount excreted), and at 7 days posttreatment, mice retained more in tissues than did rats. Over the 7-day period, in total 57.5% of the dose was absorbed in rats compared to 13.1% in mice. Total excretion of radiolabel in rats was approximately 6-fold higher than in mice (56% vs 9%), with an excretion ratio for urine to faeces of 2:1 for rats and 3.5:1 for mice. More than 90% of total excretion occurred within three days after treatment, with 70-80% of total excretion within the first 24 hrs. Absorbed radiolabel was rapidly and widely distributed in tissues, especially carcass, skin (not application site), liver, kidney, gastrointestinal tract, spleen, bladder, testes and femur, with the difference that in rats there was a shift of localization toward target organs (such as immune and haematopoietic tissues, gastrointesinal tract and testes) with time and in mice a shift from target tissues (spleen, thymus) and excretory organs (kidney and bladder) to carcass and skin. In both species, despite rapid absorption and excretion, the level of DAS or its metabolites remained relatively stable in target and other organs.

After oral administration of 2 mg DAS/kg bw to swine, DAS was rapidly absorbed (peak within 1 hr) and metabolised to 15-monoacetoxyscirpenol (MAS) and scirpentriol (SCT). Measurable amounts of DAS, MAS and SCT were present in serum for only 24 hrs (Bauer *et al.*, 1985; note: only unconjugated metabolites were determined). Most of the administered DAS was excreted in the faeces, with only low amounts in urine. The main metabolites in faeces were SCT and de-epoxy-SCT. MAS and de-epoxy-MAS were also detected, but in smaller amounts. Up to 26% of the oral dose was excreted via the vomit, either as DAS or as MAS, indicating presystemic hydrolysis in the stomach (Bauer *et al.*, 1989).

Intravenous administration of 0.5 or 1 mg DAS/kg bw to swine resulted in half-lives of 8.3-150.7 and 7.5-20.4 min, respectively, with a total body clearance of 27.3-191.7 mg/min/kg. 8 Hrs after iv administration of 0.1, 0.5 or 1 mg DAS/kg bw residues were found in liver, kidney, skeletal muscle, mesenteric lymph nodes and spleen, without dose-relationship. Highest concentrations were found in spleen and mesenteric lymph nodes (Coppock, 1984).

4.4.1.2 Metabolism

DAS is deacetylated to MAS by rat and rabbit liver carboxy esterases, the latter being more active (Ohta *et al.*, 1978).

After oral administration of 2.8 mg DAS/kg bw, three times at 7-day intervals, to male Wistar rats, DAS was not detected in urine and faeces. In urine, MAS, SCT, de-epoxy-MAS and de-epoxy SCT were detected, while faeces only contained the two de-epoxy metabolites. These data indicate that the liver microsomal enzymes are involved in the hydrolysis of DAS, and that the gastrointestinal microorganisms participate in the de-epoxidation reaction (Sakamoto *et al.*, 1986).

In bile obtained from isolated rat liver perfused with DAS, the glucuronide conjugates of MAS (the major component) and SCT were detected. No unconjugated metabolites were detected (Gareis *et al.*, 1986). It is assumed that DAS is metabolised biphasically: in phase I deacetylation (first at C4 and than at C15; Bauer *et al.* (1985)), in phase II conjugation with glucuronic acid.

Rumen microorganisms can biotransformate DAS under anaerobic conditions to MAS, SCT and to their corresponding de-epoxides, indicating presystemic metabolism and partly detoxicification of DAS. Direct de-epoxidation of DAS (resulting in de-epoxy-DAS) was not detected, suggesting that C4 deacetylation occurred prior to de-epoxidation (Swanson *et al.*, 1987a,b).

Upon anaerobic incubation with DAS, faecal microflora of rats, cattle and swine completely biotransformed DAS, primarily to de-epoxy MAS and de-epoxy SCT. By contrast, faecal microflora from chickens, horses and dogs failed to reduce the epoxide group in DAS and yielded only MAS in addition to smaller amounts of unmetabolised DAS and SCT. Intestinal (caecal) microflora from rats completely biotransformed DAS to de-epoxy MAS and de-epoxy SCT, with traces of SCT. No de-epoxy metabolites were formed upon incubation of DAS with rat faecal and intestinal microflora under aerobic conditions (Swanson *et al.*, 1988).

4.4.2 Toxic effects

4.4.2.1 Acute toxicity

When given ip or sc to male Swiss mice, the LD_{50}-values for DAS were 15.3 and 19.5 mg/kg bw, respectively (Thompson and Wannemacher, 1986). Ueno *et al.* (1973a) reported an ip LD_{50} of 14.5 mg/kg bw in male mice, with DAS inducing radiomimetic cellular injury and karyorrhexis in (amongst others) the small intestine and bone marrow. After iv administration, the LD_{50} in adult mice was 12 mg/kg bw. In newborn mice, the sc LD_{50} was 0.17 mg/kg bw (Ueno, 1983a).

In 1-day old broiler chicks, the oral LD_{50} for DAS was 3.82 mg/kg bw. Clinical signs consisted of asthenia, inappetance, diarrhoea and coma. Sublethal doses decreased body weight gain and feed consumption (Chi *et al.*, 1978). DAS also produced necrosis of lymphoid tissue and bone marrow followed by rapid cell depletion. However, after 24 h cell repletion started. Skin lesions were also seen in chicks, as well as necrosis in liver, gall bladder and gut (Hoerr *et al.*, 1981).
In swine, the iv LD_{50} for DAS was 0.376 mg/kg bw. The animals developed emesis, frequent defaecation, lethargy, posterior paresis, staggering gait and prostration by 18 h leading to death. Severe haemorrhagic necrotizing lesions and mucosal congestion involved the jejunum and ileum and large intestines, portions of which were blood-filled at necropsy. Lymphoid follicular necrosis was present in lymph nodes and spleen (Weaver *et al.*, 1978).
DAS was also tested for acute toxicity in rats (LD_{50}-values of 1.3, 0.75 and 7.3 mg/kg bw for iv, ip and oral administration, respectively), rabbits (iv LD_{50} 1 mg/kg bw) and dogs (iv LD_{50} 1.1 mg/kg bw) (Ueno, 1983a). The iv LD_{50}s originate from an investigation by Stähelin *et al.* (1968) who reported that mortality was seen at the earliest 10-48 hrs after injection. Clinical signs appeared within a few hrs and consisted of apathy, bradypnoe, cyanosis, bloody stool, diarrhoea, vomiting, tremor and tachycardia, later followed by asthenia.

In an experiment with male CD-1 mice, the ip LD_{50} of DAS was 20 mg/kg bw, and the intragastric LD_{50} was 15.5 mg/kg bw. Most deaths occurred within 36 hrs following onset of lethargy, trembling, pasty diarrhoea and cyanosis. Morphological changes in the animals that died included extensive necrosis of the lympho-haematopoietic organs and intestines (often transmural) and degeneration of testes. Sublethal doses caused time- and dose-dependent cell depletion and necrosis in lympho-haematopoietic organs (bone marrow, thymic cortex, splenic red pulp, mesenteric lymph nodes), multifocal necrosis of intestinal epithelium and necrosis of germinal epithelium followed by progressive tubule degeneration in the testes. There was also leukocytosis (due to lymphocytosis and neutrophilia), followed by leukopenia and anaemia. After sublethal exposure DAS-sensitive organs rapidly recovered except for testis where decreased weights and abnormal spermatogenesis persisted throughout the 2-week observation period (Conner *et al.*, 1986).

After dermal application to male CD-1 mice a single dose of DAS produced 0, 15, 25, 70 and 95% mortality at 5, 10, 20, 30 and 40 mg/kg bw, respectively. In this test, DAS was less toxic than T-2 (producing 100% mortality from 20 mg/kg bw onwards), but more toxic than HT-2 (0, 0, 20, 40 and 90% mortality at 5, 10, 20, 30 and 40 mg/kg bw, respectively) and 3-Ac-DON (not lethal at any dose). When given the doses in a combination of trichothecenes (T-2 + DAS, T-2 + 3-Ac-DON, T-2 + DAS + 3-Ac-DON, DAS + 3-Ac-DON), the time to reach 50% mortality decreased considerably for the combinations T-2 + DAS and T-2 + DAS + 3-Ac-DON as compared to the compounds given individually. The combinations T-2 + 3-Ac-DON and DAS + 3-Ac-DON produced similar results relative to that seen with the individual compound. Clinical observations included piloerection (except with 3-Ac-DON), conjunctivitis (with combinations), cyanosis of extremities, diarrhoea,

general CNS symptoms (at higher doses only). As to skin lesions, DAS caused necrosis followed by granulation tissue response, HT-2 caused ulceration with granulation response, and 3-Ac-DON did not produce skin lesions at all. HT-2 is the most potent in inducing necrosis in thymus, spleen and intestine, followed in descending order by DAS, T-2 and 3-Ac-DON (Schiefer *et al.*, 1986).

Following a single dermal application of 2.625 mg of DAS to 1.44 cm^2 of skin, 75% mortality was observed in male Fischer rats. In contrast, proportionate (0.75 mg to 0.42 cm^2 of skin) and higher (1.875 mg to 1 cm^2) doses were not lethal to CD-1 mice. Histopathological lesions in the rat consisted of severe necrosis and depletion or denuding of cells in the bone marrow, spleen, thymus, and small intestine. Although these lesions in the mouse were less severe or absent, the skin of mice was much more severely damaged than that of rats, with extensive edema, inflammation, epidermal sloughing, and hair follicle necrosis present (Wang *et al.*, 1996). The observed interspecies difference in acute toxicity was attributable to different patterns of absorption and tissue distribution (see 4.4.1.1): the lower absorption in mice as compared to rats may be attributable to DAS-induced skin damage, which may have served as physical barrier to absorption. The minimal damage to rat skin induced by topically applied DAS (compared with mouse skin) could have resulted in elevated penetration of the toxin, and as a consequence, greater deleterious effects on target tissues.

When given a single oral dose of 2 mg/kg bw, swine showed strong salivation (after ca. 10 min), vomiting (lasting for 30-60 min), and apathy, anorexia and posterior paresis for ca. 12 hrs, but no signs of intoxication after 24 hrs (Bauer *et al.*, 1985).

Coppock *et al.* (1985) examined swine up to 8 hrs after iv administration of 0, 0.5 or 1 mg DAS/kg bw. Observations included clinical signs, clinical chemistry and histopathology. DAS produced clinical signs of toxicity including bruxism, emesis, mahogany flushing of the skin, diarrhoea, ataxia, anuria, muscular weakness, depression and sometimes coma and death. In serum, urea nitrogen, creatinine and alanine aminotransferase were increased. Hyper- and hypoglycemia were observed, as well as moderate to marked liver glycogen depletion. Lesions developed in gastrointestinal tract, lymphoid tissues, pancreas, adrenal gland, brain, kidneys, liver, bone marrow and salivary glands. The cytotoxicity induced in these proliferative and metabolically active tissues and cells which function as specialized ionic pumps, pointed to DAS as a a potent radiomimetic substance.

Swine, cattle and dogs were given a single iv injection with DAS (0.5 or 1 mg/kg bw for swine, 0.5 mg/kg bw for dogs and cattle) and were observed for clinical signs and for effects on haematology and bone marrow. Clinical signs of intoxication included ptyalism, emesis (not in cattle), diarrhoea, ataxia, mahogany flushing of the skin (only in swine), muscular weakness and depression. The haemograms and morphological appearance of blood cells were affected by DAS treatment, and in bone marrow pathological changes were found:

moderate to severe necrosis of bone marrow haematopoietic elements, sequential increase in type and number of abnormal cells in blood (suggesting a successive destruction of haematopoietic elements), a marked left shift in the neutrophil population, metarubricytes and large platelets in blood, and replacement of lymphocytes with immature cells. The order of sensitivity to DAS was swine > dogs >> cattle (Coppock *et al.*, 1989).

4.4.2.2 Subacute toxicity

When given 1 mg DAS/kg bw by gastric intubation for 1, 4 or 8 consecutive days, male SD rats showed signs of intoxication from the third administration onwards. Reduced spontaneous movement and peribucal necrosis were observed in animals that had received eight administrations. Total proteins in plasma were decreased, while aspartate aminotransferase activity was increased (only in 8-day treated rats) (Galtier *et al.*, 1989). Effects on drug-metabolizing activity in liver, kidneys and lungs are described in 4.4.2.7.

Groups of 30 male Wistar rats received 0 or 1 mg DAS/kg bw by gastric intubation, three times weekly for a maximum of five weeks. Five rats per group were killed on day 0, 7, 14, 21, 28 or 35, and the pathological and haematological effects of DAS were studied. Treatment with DAS did not affect body weight but resulted in roughness and discoloration of the fur. Of the haematological parameters investigated, erythrocyte counts were affected the most: opposite to control rats in which the erythrocyte counts increased gradually upon maturation, in DAS-treated rats the erythrocyte counts were lower than those of controls from day 7 onwards. Haematocrit and haemoglobin values were also lower than in controls. Although the number of platelets was not different between control and treated animals, the size distribution showed a clear shift towards the larger platelets following DAS treatment. No effects were observed on (differential) leucocyte counts, mean cell volumes and mean cell haemoglobin levels. The major pathological lesions were atrophy and necrosis of the actively dividing cells of the bone marrow, thymus, spleen, lymph nodes and gastrointestinal tract. They were generally mild and noted mainly after 2-4 weeks of treatment, with some regression of effects in bone marrow, thymus and lymph nodes at week 5. According to the authors this could point to an increased detoxifying capability upon repeated exposure (Janse van Rensburg *et al.*, 1987).

In subacute experiments with male and female broiler breeders fed 0, 5, 10 or 20 mg DAS/kg diet for 2-3 weeks, the animals were observed for effects on feed consumption, body weight and oral lesions. Dose-related decreases in body weight (females only) and feed consumption were observed, as well as an increased time to consume the feed and dose-related increases in the extent of oral lesions. Feed consumption rapidly recovered after ending DAS treatment, unlike body weight. The combined effects were indicative of feed refusal that was more severe in females than males. The severity of the cytotoxic effects in the mouth increased with increased exposure duration, and the areas most sensitive to DAS treatment were associated with the salivary glands and the tip of the tongue. The lesions rapidly disappeared upon removal of DAS (although most were severe enough to produce

scarring), with exception of those of the tongue tip which took a longer recovery time or (at the higher doses) resulted in necrosis and loss of the tongue tip (Brake *et al.*, 2000).

Stähelin *et al.* (1968) limitedly described a series of experiments in which DAS was given iv to rats, dogs and monkeys for a period of 4-6 weeks or 4 months (dogs only). In these studies DAS mainly affected blood parameters (leukopenia and/or anaemia), bone marrow, spleen, liver and testes.

4.4.2.3 Carcinogenicity

In a study to investigate skin tumour induction, the skin of the back of mice was painted with 0.1-1 mg DAS, twice a week, for one year. Necrosis of the skin was found, but no tumours were detected (Lindenfelser *et al.*, 1974).

4.4.2.4 Genotoxicity

DAS was not mutagenic to *Salmonella typhimurium*, with and without metabolic activation (Wehner *et al.*, 1978). *In vitro*, DAS did not induce sister chromatid exchanges in human lymphocytes (Cooray, 1984). When tested *in vivo* in male Swiss mice after ip injection at 0.5-1 mg/kg bw, DAS caused a reduction in mitotic activity in bone marrow and an increase in structural chromosal abnormalities in somatic cells (bone marrow) and in germ cells (spermatocytes). In bone marrow, the structural abnormalities included breaks, centromeric attenuation, and endomitosis, and in spermatocytes breaks and X-Yunivalents. DAS also affected sperm morphology, by inducing increases in head abnormalities (especially amorphous and small heads) and tail abnormalities (coiled tails) (Hassanane *et al.*, 2000).

4.4.2.5 Reproductive and Developmental toxicity

In pregnant female ICR mice given a single ip injection with 0, 1, 1.5, 2, 3 or 6 mg DAS/kg bw on one of gestation days 7-11, maternal toxicity was observed at 6 (death and vaginal bleeding) and 3 (death) mg/kg bw, but not at lower doses. There was no effect on total number of implantations, but resorptions increased with dose and with day of injection (from 7-34% on gestation days 7-11 at 1 mg/kg bw, to 100% on all tested gestation days at 6 mg/kg bw). Fetal body weight of the live fetuses was significanty depressed at all doses, and was more depressed with increasing dose. A variety of fetal malformations were observed, both external (a.o. exencephaly, omphalocele, hydrocephaly, short snout, protruding tongue and meningoencephalocele) and skeletal (anomalies of the skull, sternebrae, vertebrae, vertebral centra and ribs), especially when DAS was given on day 9 of gestation. On this day external and skeletal malformations were observed at all doses (at 1 mg/kg bw even 16 and 64%, respectively) (Mayura *et al.*, 1987).

DAS is teratogenic in mice given single oral doses of 0, 1, 2, 3 or 4 mg/kg bw on gestation days 9 to 11. At 3 mg/kg bw and above, DAS reduced resorptions, and induced gross malformations (exencephaly, protruding tongue, short snout and missing tail), skeletal

abnormalities (fused ribs, missing sternum centra, missing skull bones) and soft tissue defects (cleft palate) (Gentles *et al.*, 1993).

The testis of male Lewis rats is sensitive to treatment with DAS (1x ip 1.7 mg/kg bw), as testicular weight and sperm production were reduced and the frequency of hypocellular seminiferous tubules was increased. The hypocellular tubules had few or no germinal epithelial cells and consisted almost entirely of vacuolated Sertoli cells. There was a relatively long period between exposure and the observed testicular changes, and there was no evidence of recovery. The effects reflect injury to germinal cells early in the maturation sequence. Other effects of DAS on the reproductive tract included alterations in epididymal transit times, as reflected by decreased epididymal sperm reserves occurring prior to sperm reduction (Conner *et al.*, 1990).

To examine the effects of DAS on fertility and hatchability of broiler breeders, naturally mated broiler breeders received 0, 1.25, 2.5 or 5 mg DAS/kg feed for 3 weeks from weeks 67-69 of age. A restricted feeding regimen of 154 g/bird/day was used when feeding DAS, and males and females were fed together. In a second experiment, female and male broiler breeders individually received 0, 5, 10 or 20 mg DAS/kg feed for 3 weeks from weeks 25-27 of age, according to a restricted feeding regimen of 114-125 g/bird/day depending upon age. After DAS treatment, semen was pooled from males within each treatment and used to inseminate females from each treatment.
In the first experiment, fertility was consistently improved at 5 mg/kg feed, and intermittently at 1.25 and 2.5 mg/kg feed, but the effect disappeared upon removal of DAS. Hatchability of fertile eggs was only slightly influenced at wk 68, not at other ages. In the second experiment, female-related fertility was increased at 5 and 10 mg/kg feed, and male-related fertility was decreased at 10 and 20 mg/kg feed (with the greater effect at 10 mg/kg feed). Female-related hatchability was not affected by DAS-treatment, but there was a decrease in hatchability attributed to a male effect at 10 mg/kg feed. Apparently, the quality of the semen at 10 mg/kg feed was poorer compared to 20 mg/kg feed, given the greater effect on male-related fertility at 10 mg/kg feed and the effect on hatchability. Upon necropsy, no differences in relative testes weights were found, but many treated males had small, fluid-filled cysts on the testes (Brake *et al.*, 1999).

4.4.2.6 Immunotoxicity

Intraperitoneal treatment of mice with DAS dose-dependently and significantly affected the thymus weight (reduction) and the responsiveness to sheep red blood cells (SRBC; inhibition) (Rosenstein et al., 1979).

In an *in vitro* test with mitogen-stimulated murine splenic and thymic lymphocytes, DAS reversibly inhibited the stimulation of both T and B cells and suppressed the ability to synthesize anti-SRBC antibodies. At high concentrations a direct cytostatic action was seen, but at low concentrations a stimulating action (Lafarge-Frayssinet *et al.*, 1979).

Thuvander *et al.* (1999) found that DAS effectively inhibited proliferation and immunoglobulin production (IgA, IgG and IgM) in mitogen-stimulated human lymphocytes in a dose-dependent manner with limited variation in sensitivity between individuals. However, at low levels DAS exposure could also result in enhanced proliferative responses, as well as in elevated immunoglobulin production (especially IgA). DAS was somewhat less effective than T-2 (3-4 times more potent), but more effective than DON and NIV (30-100 times less potent). Combinations of DAS with NIV or DON resulted in additive and antagonistic interactions, repectively, while for the combination of DAS with T-2 a synergistic effect could not be excluded.

4.4.2.7 Other effects

For DAS, the lowest sc dose that caused vomiting in ducklings was 0.2 mg/kg bw (Ueno, 1983a).

When applied to the shaved back skin of guinea pigs, DAS induced redness 2-10 hrs after painting (effective dose 10^{-10} mol). The dermal toxicity of DAS was comparable to that of T-2, HT-2 and type D trichothecenes, and was high as compared to NIV, 3-Ac-DON and DON (effective doses 10^{-7} - 10^{-8} mol) (Ueno, 1984).

As can be seen from Table 8 in 4.1.2.2, DAS is highly cytotoxic to cultured human cells and is a potent inhibitor of protein synthesis. Also in cultured animal cells (baby hamster kidney cells, mouse fibroblasts, mouse P-815 mastocytoma cells) DAS appeared highly cytotoxic (Stähelin *et al.*, 1968; Saito and Ohtsubo, 1974). In mitogen-stimulated human peripheral blood lymphocytes DAS has also been shown to inhibit DNA synthesis (total inhibition by 8 ng/ml, 50% inhibition by 2.7 ng/ml). When metabolic activation (by isolated rat liver cells) was present, the inhibitory effect of DAS was somewhat reduced (Cooray, 1984).

DAS has undergone clinical trials as a chemotherapeutic agent in cancer patients. Toxic effects included nausea, vomiting, hypotension, neurological symptoms, chills, fever and diarrhoea. Also the haematopoietic system appeared extremely sensitive, showing severe myelosuppression (WHO, 1990). Because of its toxicity, this use of DAS was stopped (Eriksen and Alexander, 1998).

Mice treated orally with 3 mg DAS/kg bw for 2 days before ip inoculation with *Listeria monocytogenes* showed increased mortality and splenic *Listeria* counts. The thymus weight was reduced, and lymphocytes were depleted from the thymus cortex and from splenic lymphoid follicles and periarteriolar lymphoid sheaths. A single dose of 4 mg DAS/kg bw on day 6 before challenge exposure did not affect mortality. Mice treated with DAS and subsequently inoculated with *Listeria* had higher levels of neutrophil populations than *Listeria*-infected control mice (Ziprin and Corrier, 1987).

After infection of human epidermoid carcinoma no.2 (HEp-2) cells with herpes simplex virus type 2, DAS inhibited virus replication (EC_{50} of 2.3 ng/ml) by blocking viral protein synthesis. The antiviral activity of DAS was greater than that of NeoSol (EC_{50} of 52 ng/ml) but somewhat less than that of T-2 (EC_{50} of 1.6 ng/ml) (Okazaki *et al.*, 1988).

In the liver of rats, intubation with 1 mg DAS/kg bw for up to 8 days resulted in significant decreases in microsomal and cytosolic proteins, in microsomal cytochrome P-450 content and, consistent with the latter, in cytochrome P-450-dependent monooxygenase activities. Hepatic microsomal cytochrome b5 content was not affected, nor were the conjugating enzymes, except for *p*-nitrophenol glucuronyltransferase activity which was increased. DAS-treatment had no effect on enzyme activities in kidneys and lungs (Galtier *et al.*, 1989).

4.5 3-Acetyl-deoxynivalenol (3-Ac-DON)

4.5.1 Toxicokinetics

Very little data exist on the toxicokinetics of 3-Ac-DON. 3-Ac-DON is deacetylated to DON by rabbit liver carboxy esterases (Ohta *et al.*, 1978).

4.5.2 Toxic effects

4.5.2.1 Acute toxicity

When given ip or sc to male Swiss mice, the LD_{50}-values for 3-Ac-DON were 54 and 59 mg/kg bw, respectively (Thompson and Wannemacher, 1986). Ueno (1983a) reported LD_{50}-values for 3-Ac-DON in mice (49 and 34 mg/kg bw for ip and oral administration, respectively), dogs (sc LD_{50} >1 mg/kg bw), and ducklings (sc LD_{50} 37 mg/kg bw).

To study the pathology of single oral doses of 3-Ac-DON, Schiefer *et al.* (1985) examined weanling male Crl:CD1 mice up to 96 hrs after intragastric administration of 0, 5, 10, 20 or 40 mg/kg bw 3-Ac-DON. Signs of toxicity were seen at 20 and 40 mg/kg bw and 40 mg/kg bw produced death by 24 hrs, with all animals dying by 96 hrs. Lesions were observed in the duodenal crypts, thymus and spleen, but not in stomach, pancreas, liver or heart. In the affected organs and tissues, effects were seen on the dividing cells (reduction in mitotic activity in duodenum, increase in necrotic cells in duodenum, tymus and spleen, and nuclear necrosis characterized by nuclear pyknosis and karyorrhexis), with the highest intensity at 40 mg/kg bw and less at 20 and 10 mg/kg bw.

After dermal application of 3-Ac-DON to male CD-1 mice at single doses of 5, 10, 20, 30 or 40 mg/kg bw, no animals died. In this test, 3-Ac-DON was less toxic than T-2 (producing 100% mortality from 20 mg/kg bw onwards), DAS (0, 15, 25, 70 and 95% mortality at 5, 10,

20, 30 and 40 mg/kg bw, respectively) and HT-2 (0, 0, 20, 40 and 90% mortality, respectively). When given the doses in a combination of trichothecenes (T-2 + DAS, T-2 + 3-Ac-DON, T-2 + DAS + 3-Ac-DON, DAS + 3-Ac-DON), the time to reach 50% mortality decreased considerably for the combinations T-2 + DAS and T-2 + DAS + 3-Ac-DON as compared to the compounds given individually. The combinations T-2 + 3-Ac-DON and DAS + 3-Ac-DON produced similar results relative to that seen with the individual compound. Clinical observations included piloerection (except with 3-Ac-DON), conjunctivitis (with combinations), cyanosis of extremities, diarrhoea, general CNS symptoms (at higher doses only). As to skin lesions, 3-Ac-DON did not produce skin lesions at all, whereas DAS caused necrosis followed by granulation tissue response and HT-2 caused ulceration with granulation response. HT-2 is the most potent in inducing necrosis in thymus, spleen and intestine, followed in descending order by DAS, T-2 and 3-Ac-DON (Schiefer *et al.*, 1986).

4.5.2.2 Subacute toxicity

Groups of weanling male Crl:CD1 mice were given 3-Ac-DON in the diet at concentrations of 0, 2.5, 5 or 10 mg/kg feed for 7, 14 or 21 days. Observations included body weight, feed consumption, haematology, immunological function, gross examination and microscopic examination of thymus, spleen, stomach and duodenum. Feed consumption was dose-relatedly reduced in the first week of treatment but not thereafter. Body weights were also depressed (in the first week at 10 mg/kg feed, in the second week at 5 and 10 mg/kg feed), but not in the third week. The primary immune response against SRBC was not affected after 7 or 14 days, but was dose-relatedly enhanced after 21 days. Haematology was not affected, and no macroscopic or microscopic lesions were detected (Kasali *et al.*, 1985).

In a 48-day feeding trial, weanling male Swiss mice were examined for the effects of 0, 2.5, 5, 10 or 20 mg 3-Ac-DON/kg feed on body weight, feed and water consumption, and organ weights of spleen, thymus, liver and kidney. At 20 mg/kg feed, mice became dull and depressed-looking, with rough hair coats initially. Feed consumption and body weight gain were reduced at 10 and 20 mg/kg bw during the first few days of treatment, but rapidly returned to normal. Of the organ weights, only liver weight was increased at 20 mg/kg feed (Kasali *et al.*, 1985).

Groups of 5-6 swine were exposed for 21 days to feed containing 0 or 2 mg/kg 3-Ac-DON, with and without 6 mg DON/kg feed. DON-fed animals showed lower daily feed consumption and daily weight gain and poorer feed efficiency than animals fed DON-free diets, particularly during the first 7 days. Over time, however, the differences decreased. There was no statistically significant effect of adding 3-Ac-DON to the diet, although with 3-Ac-DON the decreases in feed consumption, weight gain and feed efficiency were somewhat less compared with DON-free and DON-fed controls (Rotter *et al.*, 1992).

4.5.2.3 Genotoxicity

3-Ac-DON was not mutagenic to *Salmonella typhimurium* (Wehner *et al.*, 1978) but induced chromosomal aberrations in Chinese hamster V79 cells *in vitro* (Hsia *et al.*, 1988).

4.5.2.4 Immunotoxicity

When tested for its *in vitro* effect on mitogen-induced lymphocytic blastogenesis in rat or human peripheral blood lymphocytes, 3-Ac-DON inhibited blastogenesis by 50% at concentrations of 450 and 1060 ng/ml, respectively. DON was more effective, with 50% inhibitory concentrations of 90 and 220 ng/ml, respectively (Atkinson and Miller, 1984).

Depending on dose and time, 3-Ac-DON and other trichothecenes like T-2, DON, NIV and 15-Ac-DON, could inhibit or superinduce cytokine production and mRNA expression in murine spleen CD4+ T cells. The rank order of superinduction was T-2 > DON, NIV > 15-Ac-DON > 3-Ac-DON (Ouyang *et al.*, 1995).

In an *in vitro* test with human peripheral blood mononuclear cells, 3-Ac-DON significantly and dose-dependently inhibited the T and B cell proliferation. This effect was also observed for T-2, FusX, NIV and DON, but hardly or not at all for α- and β-zearalenol. For T-2, NIV and DON, the immunomodulatory effect on mononuclear cells also included a suppression in natural killer cell activity and an inhibition in antibody-dependent cellular cytotoxicity reaction, but this was not investigated for 3-Ac-DON (Berek *et al.*, 2001).

4.5.2.5 Other effects

For 3-Ac-DON, the lowest sc dose that caused vomiting in ducklings was 13.5 mg/kg bw. When tested in dogs the lowest effective dose after iv administration was 0.2 mg/kg bw. These effective doses were comparable to those for DON (13.5 and 0.1 mg/kg bw, respectively) (Ueno, 1983a).

When applied to the shaved back skin of guinea pigs, 3-Ac-DON induced redness 2-10 hrs after painting (effective dose 10^{-8} mol). Compared to T-2, HT-2, DAS and type D trichothecenes (effective doses 10^{-10} - 10^{-11} mol), 3-Ac-DON was low in dermal toxicity (Ueno, 1984).

As can be seen from Table 8 in 4.1.2.2, 3-Ac-DON is cytotoxic to cultured cells and possesses protein synthesis inhibiting activity.

4.6 15-Acetyl-deoxynivalenol (15-Ac-DON)

4.6.1 Toxicokinetics

No data were available on the toxicokinetics of 15-Ac-DON.

4.6.2 Toxic effects

4.6.2.1 Acute toxicity

When given orally or ip to weanling female B6C3F1 mice, the LD_{50}-values for 15-Ac-DON were 34 and 113 mg/kg bw, respectively. Death occurred within 48 hrs, with 15-Ac-DON inducing extensive necrosis of the gastrointestinal tract (the crypt epithelial cells in the small and large intestine in particular) and of the haematopoietic and myelopoietic elements in the bone marrow, lymphoid necrosis in the spleen and thymus, as well as focal lesions in the kidney (tubular necrosis). The minimum 15-Ac-DON doses required to cause these lesions were 160 mg/kg bw ip and 40-100 mg/kg bw orally (Forsell *et al.*, 1987).

4.6.2.2 Subacute toxicity

Groups of 5-6 swine were exposed for 20 days to feed containing 0 or 2 mg/kg 15-Ac-DON, with and without 6 mg DON/kg feed. DON-fed animals showed lower daily feed consumption and daily weight gain and poorer feed efficiency than animals fed DON-free diets, particularly during the first 7 days. Over time, however, the differences decreased. There was no effect of adding 15-Ac-DON to the diet, i.e. the effects seen in 15-Ac-DON-fed animals mirrored those seen in the DON-free and DON-fed controls (Rotter *et al.*, 1992).

Groups of 10 female weanling B6C3F1 mice were administered 0.5, 2 or 5 mg 15-Ac-DON/kg diet for 56 days. An additional group of 20 animals served as controls. Observations included feed intake, body weight, organ weights (brain, liver, kidney, thymus and spleen) and blood clotting function. Statistically significant effects were only observed at 5 mg/kg. Reduced feed intake was observed from day 44 onwards, but reduced body weight gain already from day 16 onwards. The body weight effect was associated with reduced feed conversion efficiency. Absolute liver, kidney and spleen weights were reduced, while relative spleen and kidney weights were increased. When expressed as percentages of brain weight, 15-Ac-DON at 5 mg/kg caused lower liver and kidney weights than could be attributed to concurrent losses in body weight. Treatment with 15-Ac-DON decreased bleeding times, but other measurements of clotting function indicated no effect of 15-Ac-DON (Pestka *et al.*, 1986).

4.6.2.3 Immunotoxicity

15-Ac-DON inhibited blastogenesis in cultured human lymphocytes. It produced a 50% inhibition of [^{3}H]thymidine uptake in mitogen-stimulated human lymphocytes at a

concentration of 240 ng/ml. Other trichothecenes like T-2, HT-2, FusX, NIV and DON produced 50% inhibition in this system at concentrations of 1.5, 3.5, 18, 72 and 140 ng/ml, respectively. These results indicate that the lymphotoxicity of trichothecenes is related to the C4 substituent: acetyl>hydroxyl>hydrogen. Acetylation at C15 slightly decreases lymphotoxicity (Forsell and Pestka, 1985; Forsell *et al.*, 1985).

Depending on dose and time, 15-Ac-DON and other trichothecenes like T-2, DON, NIV and 3-Ac-DON, could inhibit or superinduce cytokine production and mRNA expression in murine spleen CD4+ T cells. The rank order of superinduction was T-2 > DON, NIV > 15-Ac-DON > 3-Ac-DON (Ouyang *et al.*, 1995).

4.6.2.4 Other effects

Groups of three swine were given single oral or ip doses of 25, 50, 75, 100 or 200 µg/kg bw 15-Ac-DON or DON. The minimum 15-Ac-DON dose at which emesis occurred was 75 µg/kg bw for both routes of administration. 15-Ac-DON was, however, more effective in inducing vomiting when administered ip than orally: after ip administration more pigs were affected (9/15 ip compared to 3/15 orally), and increased dosage was associated with decreased average time to vomition, increased duration of emesis and increased average number of vomitions. For DON comparable results were found, with DON being slightly more effective than 15-Ac-DON (minimum emetic dose for DON 50 µg/kg bw for both routes of administration) (Pestka *et al.*, 1987).

When tested in the chick embryotoxicity screening test, in which mortality of chicken embryos was measured after hatching eggs for 22 days, 15-Ac-DON was slightly less toxic than DON (LD_{50}-values of 10.5 and 6.05 µg/egg, respectively). Both 15-Ac-DON and DON were considerably less toxic than HT-2 (LD_{50}-value 0.73 µg/egg) (Prelusky *et al.*, 1989). When two trichothecenens were combined (15-Ac-DON + DON, 15-Ac-DON + HT-2, DON + HT-2) at levels of LD_{10}, LD_{20} and LD_{40} for each, the combined toxicity was found to be additive (Rotter *et al.*, 1991).

4.7 Neosolaniol (NeoSol)

4.7.1 Toxicokinetics

Very little data exist on the toxicokinetics of NeoSol. In contrast to DAS, NeoSol (= 8-OH-DAS) is not deacetylated by rat and rabbit liver carboxy esterases. Apparently, the hydrophylic nature of the substituent at C8 interferes with the esterase activity (Ohta *et al.*, 1978).

4.7.2 Toxic effects

Data on toxic effects of NeoSol are rather scarce. Besides, most data available lack details because they have been reported in reviews or in abstract-form.

When given ip or sc to male Swiss mice, the LD_{50}-values for NeoSol were 14.8 and 9.7 mg/kg bw, respectively (Thompson and Wannemacher, 1986).
In 1-day old broiler chicks, the oral LD_{50} for NeoSol was 24.87 mg/kg bw. Clinical signs consisted of asthenia, inappetance, diarrhoea and coma. Sublethal doses decreased body weight gain and feed consumption (Chi *et al.*, 1978).
Ueno *et al.* (1973a) reported an ip LD_{50} of 14.5 mg/kg bw in male mice, with NeoSol inducing radiomimetic cellular injury and karyorrhexis in (amongst others) the small intestine and bone marrow. A slightly higher ip LD_{50} of 17.8 mg/kg bw was reported by Yoshizawa *et al.* (1985).

The lowest sc dose causing vomiting in ducklings was 0.1 mg NeoSol/kg bw. NeoSol was as effective as T-2 and HT-2 (Ueno, 1983a). In cats, NeoSol caused vomiting 2 h after sc administration followed by diarrhoea and anorexia (Ueno et al., 1973b).

When applied to the shaved back skin of guinea pigs, NeoSol induced redness 2-10 hrs after painting (effective dose 10^{-9} mol). NeoSol was of intermediate dermal toxicity when compared to T-2, HT-2, DAS and type D trichothecenes (effective doses 10^{-10} - 10^{-11} mol) and NIV, 3-Ac-DON and DON (effective doses 10^{-7} - 10^{-8} mol) (Ueno, 1984).

Repeated sc administration of NeoSol to cats induced marked decrease of white blood cells and ataxia in the hind legs. On autopsy, extensive meningeal bleeding and lung haemorrhage (Ueno *et al*., 1973b).
Early after injection of NeoSol in mice, a rapid increase of leucocytes and lymphocytes was seen, accompanied by increased β-globulin and decreased γ-globulin. On autopsy, cellular degeneration and karyorrhexis were marked in bone marrow and small intestine (Ueno *et al*., 1973b).

NeoSol is highly cytotoxic to cultured cells and possesses protein synthesis inhibiting activity (see Table 8 in 4.1.2.2).

After infection of human epidermoid carcinoma no.2 (HEp-2) cells with herpes simplex virus type 2, NeoSol inhibited virus replication (EC_{50} of 52 ng/ml) by blocking viral protein synthesis. The antiviral activity of NeoSol was less than that of DAS (EC_{50} of 2.3 ng/ml) and T-2 (EC_{50} of 1.6 ng/ml) (Okazaki *et al.*, 1988).

5. Evaluation

5.1 NIV

From the data available it can be concluded that absorption of NIV is fast and extensive, and excretion is mainly via faeces. After adaptation, the intestinal microflora of swine and rats can de-epoxidise NIV. NIV is not de-epoxidated in chickens, where an unknown metabolite is formed in varying amounts.

After oral administration, NIV is acutely toxic to very toxic with LD_{50}-values of 19.5 mg/kg bw in rats and 38.9 mg/kg bw in mice.

Almost all repeated dose toxicity studies were performed with mice, using diets prepared by mixing basal diet with rice powder artificially made mouldy by NIV-producing *F. nivale* Fn 2B (claimed not to produce other trichothecenes on rice, with no FusX detectable). Only a few studies with rats have been reported. From these studies and from comparison of the oral LD_{50}-values it appears that rats are more susceptible to NIV than mice.
Major toxic manifestations in subacute, subchronic and chronic toxicity experiments with NIV in mice were reduced body weight gain and reduced feed efficiency, organ weight changes (but without histopathology findings) and haematotoxicity (bone marrow toxicity, leukopenia and/or erythrocytopenia, depending on study duration). In a 2-year feeding trial NIV did not affect tumour development in female mice.
In subacute feeding studies with swine NIV caused mild pathological changes in gastrointestinal tract, kidney and spleen, without vomiting or affecting leucocyte counts, body weight gain and food consumption.

IARC (1993) concluded that there is inadequate evidence in experimental animals for the carcinogenicity of NIV. No human data are available, and overall IARC concluded that NIV is not classifiable as to its carcinogenicity to humans (Group 3).

NIV is a weak inducer of chromososal aberrations in mammalian cells *in vitro* and from indicator tests it appears that NIV has the potential to induce DNA-damage. However, the available information is too limited (a.o. no gene mutation tests) to evaluate the genotoxic potential of NIV.

In mice, NIV is embryotoxic and fetoxic but not teratogenic. Data from other species are lacking, as well as data on reproductive toxicity.

NIV exerts antiviral and immunotoxic activity. Depending on dose and exposure regimen, it can be both immunosuppressive and immunostimulatory.

Taking into account the above, as well as its cytotoxicity, dermal toxicity, protein and DNA synthesis inhibition and potential to induce vomiting, the mechanism of action of NIV is presumably similar to that of other type A and B trichothecenes, given also the structural similarities.

In Table 9 the oral N(L)OAELs[1] for NIV as found in the studies described in 4.2.2.2-7 are given.

Table 9 Oral N(L)OAELs for NIV in investigated animal species

	Study	Critical effect	NOAEL	LOAEL
Repeated dose toxicity				
rat	15-30 days (gavage)	increased liver and spleen weights	0.4 mg/kg bw	2 mg/kg bw
rat	2-4 weeks (diet)	variably affected organ weights, induction of drug metabolizing enzymes	-	6 mg/kg feed
rat	8 weeks (diet)	reduced body weight gain, weak erythrocytopenia, gut and bone marrow damage	10 mg/kg feed	50 mg/kg feed
mouse	24 days (diet)	erythrocytopenia, slight leukopenia, bone marrow damage	10 mg/kg feed	30 mg/kg feed
mouse	4-12 weeks (diet)	reduced body weight gain, increased alkaline phosphatase activity	-	6 mg/kg feed
mouse	6 months-1 year (diet)	reduced body weight gain and feed efficiency, leukopenia (after 1 year)	-	6 mg/kg feed (0.68-0.76 mg/kg bw)
mouse	2 year (diet)	reduced body weight gain and feed efficiency	-	6 mg/kg feed (0.66 mg/kg bw)
swine	3 weeks (diet)	mild gastrointestinal, kidney and splenic lesions, reduced spleen cell numbers	-	2.5 mg/kg feed (0.1 mg/kg bw)
chicken	20 days (diet)	gizzard erosions, reduced body weight gain and feed consumption	-	3 mg/kg feed
Developmental toxicity				
mouse	throughout gestation (diet)	intrauterine growth retardation	6 mg/kg feed	12 mg/kg feed

[1] NOAEL = no-observed-adverse-effect-level; LOAEL = lowest-observed-adverse-effect-level

	Study	Critical effect	NOAEL	LOAEL
mouse	day 7-15 of gestation (gavage)	intrauterine growth retardation	1 mg/kg bw	5 mg/kg bw
Immunotoxicity				
mouse	4-8 weeks (diet)	elevated IgA levels and pathological changes	-	6 mg/kg feed

Overall, the general toxicity and immunotoxicity appear the most sensitive effects of NIV in the tested animal species, with a LOAEL of approximately 0.7 mg/kg bw/day in 4-week to 2-year feeding studies with mice and a (marginal) LOAEL of 0.1 mg/kg bw/day in a 3-week dietary study with swine. Given the time period tested (4 weeks to 2 years) and the effects observed (both general toxicity and immunotoxicity), the LOAEL from the mouse study is considered most appropriate to be used as basis for the derivation of a TDI for NIV.
To derive a TDI, the LOAEL is to be divided by uncertainty factors to cover for interspecies (factor 10) and intraspecies (factor 10) differences. In addition, because of the use of a LOAEL and some deficiencies in the data set, an extra uncertainty factor of 10 is necessary. Applying the overall uncertainty factor of 1000 to the LOAEL of 0.7 mg/kg bw/day, the resulting TDI is 0.7 µg/kg bw. This derived TDI is not a full TDI but a temporary one, due to deficiences in the data set. It is to be noted that the tTDI derived here is in concordance with the tTDI established for NIV by the SCF (see chapter 1). Comparing the (marginal) LOAEL of 0.1 mg/kg bw/day from the 3-week study in swine with the tTDI, a margin of safety of approximately 140 can be derived. This margin of safety is considered sufficient, given also that the physiology of swine resembles that of humans. Hence, there is no reason to deviate from the tTDI as also derived by the SCF.

5.2 FusX

From the few data available on the toxicokinetics of FusX it appears that FusX is rapidly absorbed and excreted, and that FusX is deacetylated to NIV.

After oral administration, FusX is acutely very toxic with LD_{50}-values of 4.4 mg/kg bw in rats and 4.5 mg/kg bw in mice.

Only a few repeated dose toxicity studies with FusX have been reported. The description of most of these studies is rather limited. Besides, in the oral studies with FusX (isolated from culture filtrates of *F. nivale* (Fn 2B)) only male rats were tested. In these studies FusX caused intrahepatic bile duct hyperplasia, atypical hyperplasia in the gastric and intestinal mucosa and hypoplasia and atrophy of the bone marrow, thymus and spleen. FusX did not affect

tumour development in a 2-year feeding trial, but it is to be noted that it reduced growth rate and increased the liability to pulmonary infections, resulting in decreased survival.

IARC (1993) concluded that there is inadequate evidence in experimental animals for the carcinogenicity of FusX. No human data are available, and overall IARC concluded that FusX is not classifiable as to its carcinogenicity to humans (Group 3).

In vitro, FusX does not induce gene mutations in bacteria and mammalian cells but is weakly clastogenic in the latter. As to DNA-damage, the results are equivocal. The available information is too limited to evaluate the genotoxic potential of FusX.

In mice, FusX affects pregnancy, either by inhibition of implantation, or embryonal and fetal death with resulting absorption and abortion, or retardation of fetal growth. FusX is not teratogenic. Data from other species are lacking, as well as data on reproductive toxicity.

FusX exerts immunosuppressive and antiviral activity.

Taking into account the above, as well as its cytotoxicity, dermal toxicity, protein and DNA synthesis inhibition and potential to induce vomiting and diarrhoea, the mechanism of action of FusX is presumably similar to that of other type A and B trichothecenes, given also the structural similarities.

Given the deficiencies in the data package for FusX, in particular the limited number of oral studies and their shortcomings, it is not possible to derive a (t)TDI. Due to lack of sufficient data on the toxicity of FusX, also the Nordic Working Group did not set a (t)TDI for this trichothecene (Eriksen and Alexander, 1998).

5.3 DAS

After oral administration, DAS is rapidly and extensively absorbed and widely distributed in tissues and organs, amongst which the lympho-haematopoietic organs and intestines. In rats and mice, excretion is mainly via urine, while in swine it is mainly via faeces and vomit. After topical administration, rats and mice have different patterns of absorption, tissue distribution and excretion, with rats having a greater absorption and higher concentrations in target and other organs.
Metabolism of DAS firstly involves hydrolysis/deacetylation to MAS and SCT, which is then followed by glucuronidation and de-epoxidation. The latter takes place by the (anaerobic) intestinal and faecal microflora of rats, cattle and swine. Chickens, horses and dogs lack the necessary microflora for epoxide reduction. In ruminants, the ruminal flora is also able to hydrolyse/deacetylate and de-epoxydate DAS.

Orally, DAS is acutely very toxic with LD_{50}-values of 7.3 mg/kg bw in rats, 15.5 mg/kg bw in mice and 4-5 mg/kg bw in young chickens. Rats are more sensitive than mice to the systemic effects of topically applied DAS, whereas mice are more sensitive to skin damage.

Only a few studies with repeated administration of DAS are available. These (subacute) studies, however, were not full toxicity studies as they addressed only a few effects, and some only used one dose level. The major toxic effects observed in the subacute studies with DAS resembled those seen in the acute studies and consisted of haematologic changes (leukopenia and/or anaemia), cell depletion and necrosis of the lympho-haematopoietic organs (bone marrow, thymus, spleen, lymph nodes) and intestines and degeneration of testes. Vomiting was observed in swine, dogs and poultry, and the latter also showed feed refusal, reduced body weight gain and mouth lesions.

In vitro, DAS does not induce gene mutations in bacteria or sister chromatid exchanges in human lympohocytes. *In vivo*, DAS affects mitotic activity in bone marrow and induces chromosomal abnormalities in both somatic (bone marrow) and germ cells (spermatocytes). It also affects sperm morphology. Hence, DAS is a genotoxic substance.

In mice, DAS is fetotoxic and teratogenic. Data from other species are lacking, and no data on reproductive toxicity in laboratory animals are available. In poultry, low doses of DAS improved female-related fertility, presumably because of enhanced spermatozoal storage within the oviduct. Conversely, higher doses of DAS decreased male-related fertility, presumably by a direct toxic effect on the testes as was shown for rats and mice, where DAS induced testicular changes (degeneration, reduced weight and abnormal spermatogenesis).

DAS exerts immunotoxic and antiviral activity.

Taking into account the above, as well as its cytotoxicity, dermal toxicity, protein and DNA synthesis inhibition and potential to induce vomiting, the mechanism of action of DAS is presumably similar to that of other type A and B trichothecenes, given also the structural similarities.

In total, the data package available for DAS is too limited (e.g. only a few oral studies with shortcomings, no studies of long duration) to allow derivation of a (t)TDI. Besides, DAS is genotoxic.

5.4 3-Ac-DON

Due to the limited data set available on 3-Ac-DON it is not possible to conclude on its toxicokinetic and toxicological properties. It can only be established that after oral

administration 3-Ac-DON is acutely toxic (LD_{50} in mice is 34 mg/kg bw), with 3-Ac-DON affecting the dividing cells of thymus, spleen and intestines, and that 3-Ac-DON exerts immunotoxic activity (both immunosuppressive and immunostimulating). The few parameters that were investigated in the repeated dose toxicity studies available for 3-Ac-DON seem to indicate a potential for initial feed refusal and reduced body weight gain. Presumably, the mechanism of action of 3-Ac-DON is similar to that of other type A and B trichothecenes given the structural similarities and the toxic effects of 3-Ac-DON seen in the reported studies.

Given the deficiencies in the data package, no (t)TDI can be derived for 3-Ac-DON.

5.5 15-Ac-DON

Like for 3-Ac-DON, the data set available on 15-Ac-DON does not allow to conclude on its toxicokinetic and toxicological properties. From the limited data available it appears that after oral administration 15-Ac-DON is acutely toxic (LD_{50} in mice is 34 mg/kg bw), with 15-Ac-DON affecting the dividing cells of bone marrow, thymus, spleen and intestines, that 15-Ac-DON can affect feed consumption and body weight gain, and that 15-Ac-DON exerts immunotoxic activity (both immunosuppressive and immunostimulating). Presumably, the mechanism of action of 15-Ac-DON is similar to that of other type A and B trichothecenes given the structural similarities and the toxic effects of 15-Ac-DON seen in the reported studies.

The data package available for 15-Ac-DON is too limited to derive a (t)TDI for 15-Ac-DON.

5.6 NeoSol

The available data do not allow to conclude on the toxicokinetic and toxicological properties of NeoSol. Presumably, the mechanism of action of NeoSol is similar to that of other type A and B trichothecenes given the structural similarities and the toxic effects of NeoSol seen in the few reported studies.

The data package available for NeoSol is far too limited to derive a (t)TDI for NeoSol.

6. Conclusions

Of all known trichothecenes only a few are believed to be of importance with respect to their actual presence in crops, animal feed and human foodstuffs. These trichothecenes are DON, NIV, DAS and T-2 and their derivatives HT-2, 3-Ac-DON, 15-Ac-DON, NeoSol and FusX. It is very important to assess the potential health effects of these most commonly occurring trichothecenes and to derive TDIs where possible, because of the worldwide trade in cereals, feeds and foods that more and more are globally contaminated with mycotoxins, including trichothecenes.

Recently, an evaluation of the toxicity of DON, T-2 and HT-2 by the SCF and JECFA has resulted in (t)TDIs for these substances (1 µg/kg bw for DON and 0.06 µg/kg bw for the sum of T-2 and HT-2). This report therefore focussed on the toxicity of six other trichothecenes, i.e. NIV, FusX, DAS, 3- and 15-Ac-DON and NeoSol, in order to see if it was possible to derive (t)TDIs for these substances as well (which in the mean time was already done for NIV by the SCF).

Although for some of the six trichothecenes studied the available data package was rather limited, overall it seems that the toxic effects observed for NIV, FusX, DAS, 3- and 15-Ac-DON and NeoSol fit quite well the profile of toxic effects of trichothecenes in general. The most important observed effects in our evaluation were haematotoxicity, immunotoxicity, embryo- and fetotoxicity, reductions in body weight gain and food consumption, vomiting and, for some, (intrinsic) genotoxicity. However, the limited toxicology data available for FusX and DAS, and especially for 3- and 15-Ac-DON and NeoSol, did not allow us to derive a (t)TDI for these five trichothecenes. Only for NIV we derived a tTDI of 0.7 µg/kg bw. Due to deficiences in the data set a temporary and not a full TDI was derived.

Given that NIV and the other five trichothecenes evaluated in this report posses the same basic chemical structure of trichothecenes, they presumably share common mechanisms of action. This would favour the establishment of a group TDI, which is also supported by the fact that from feed and food analyses it appears that in the same food commodity there is often co-occurrence of trichothecenes (in particular DON and NIV) and other *Fusarium* mycotoxins like zearalenone and fumonisins (see chapter 3). Hence, in practice animals and humans will be exposed to more than one trichothecene (and even to more mycotoxins than just trichothecenes as well).
The SCF recently performed a group evaluation of DON, NIV, T-2 and HT-2. They concluded that the available data, while limited, did not support the establishing of a group TDI for the four trichothecenes evaluated, in particular because “synergism has not been observed”. This might be a correct conclusion, but it should be noted that in order to assess

the appropriateness of a group TDI for trichothecenes, much more information is needed on the actual mechanism of action of the individual trichothecenes, on the similarity of the critical effect that is crucial for the derivation of a (t)TDI, on the relative potency of the various trichothecenes to induce this effect and on the possible additivity or synergism after combining two or more trichothecenes. Also, more information is needed on the toxic effects induced by trichothecenes at the low levels usually found in contaminated feed and food.

Of the six trichothecenes under study in this report, NIV is the one most often found in cereal grains and animal feed, often together with DON and zearalenone. NIV is also found in human food, although only in cereals and processed grains and not in food derived from animals given contaminated feed. Due to fast and extensive metabolism and excretion, it is indeed unlikely that NIV, as well as other trichothecenes, will accumulate in exposed animals and will be transferred into meat, milk and eggs.
Although small amounts of FusX occur together with NIV, FusX itself has rarely been reported to occur in cereal grains and feeds and foods. Also occasionally, the occurrence of DAS, 3-Ac-DON, 15-Ac-DON or NeoSol in these commodities has been reported. It should be noted, though, that in most monitoring programs these five trichothecenes likely have not been routinely included. Hence, in order to assess whether or not they are a potential threat to animals or humans, it is recommended to pay more attention to FusX, DAS, 3- and 15-Ac-DON and NeoSol in routine monitoring programs. Analytical methods for these compounds are available. In case it turns out that these trichothecenes are regularly found at considerable levels in cereal grains, feeds or foods, further consideration has to be given on how to deal with the limited data available on the toxicology of these compounds.

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Appendix 1 Mailing list

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