



Please note that this opinion, published on 2 February 2007, replaces the earlier version published 17 June 2004.

The following item have been changed:

Page 18, 9. Human dietary exposure,

“the TDI of 1 mg/kg b.w./day” has been replaced by “the TDI of 1 µg/kg b.w./day”.

Opinion of the Scientific Panel on Contaminants in the Food Chain on a request from the Commission related to Deoxynivalenol (DON) as undesirable substance in animal feed

(Question N° EFSA-Q-2003-036)

Adopted on 2 June 2004

SUMMARY

Deoxynivalenol (DON, vomitoxin) is a mycotoxin produced by several field fungi, including *Fusarium graminearum* and *Fusarium culmorum*. The toxin is common in cereals and grains, particularly in wheat, barley and maize. Co-occurrence with other *Fusarium* toxins, including zearalenone, nivalenol (and other trichothecenes) as well as the group of fumonisins, is regularly observed. In domestic animals, exposure to DON results in loss of appetite, feed refusal and vomiting, accompanied with a decrease in weight gain. At low doses decreased feed intake seems to be attributable to the stimulation of the synthesis of pro-inflammatory cytokines under practical conditions, whilst at higher concentrations vomiting is triggered by the interaction of DON with serotonergic and dopaminergic receptors. Pigs have been identified as the most sensitive animals species regarding these adverse effects. However, at present the available data on exposure via feedingstuffs are incomplete, and no safe intake levels for pigs could be deduced from these data. Following absorption, DON is rapidly metabolised by de-epoxidation and glucuronidation, yielding less toxic products. There is no evidence for teratogenicity and genotoxicity of DON and its metabolites, neither in laboratory animal species nor in target animals. Transfer of DON and its metabolites into edible tissues, milk and eggs is very low. Thus, products of animal origin do not contribute significantly to human exposure.

Key words: Deoxynivalenol, animal feeds, toxicity, tissue residues.

TABLE OF CONTENTS

SUMMARY.....	2
TABLE OF CONTENTS.....	3
BACKGROUND.....	4
1. General Background.....	4
2. Specific Background	5
TERMS OF REFERENCE.....	5
ASSESSMENT	6
1. Introduction	6
2. Analysis	7
3. Current legislation	8
4. Occurrence of deoxynivalenol in feed materials.....	8
5. Estimating deoxynivalenol exposure of farm livestock	10
6. Adverse effects of DON.....	11
6.1. Mode of action	11
6.2. Toxic effects in pigs.....	12
6.3. Toxic effects in cattle	13
6.4. Toxic effects in sheep.....	13
6.5. Toxic effects in poultry.....	14
6.5. Toxic effects in horses.....	15
6.6. Toxic effects in rabbits.....	15
6.7. Toxic effects in dogs.....	15
6.8. Toxic effects in cats.....	16
7. Toxicokinetics.....	16
7.1. Toxicokinetics in pigs	16
7.2. Toxicokinetics in cattle	16
7.3. Toxicokinetics in sheep.....	17
7.4. Toxicokinetics in poultry	17
8. Carry-over and residues	17
9. Human dietary exposure	18
CONCLUSIONS.....	18
RECOMMENDATIONS	19
DOCUMENTATION PROVIDED TO EFSA	19
REFERENCES.....	20
SCIENTIFIC PANEL MEMBERS.....	29
ACKNOWLEDGEMENT	29
ANNEX.....	30

BACKGROUND

3. General Background

Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed¹ replaces since 1 August 2003 Council Directive 1999/29/EC of 22 April 1999 on the undesirable substances and products in animal nutrition².

The main modifications can be summarised as follows:

- extension of the scope of the Directive to include the possibility of establishing maximum limits for undesirable substances in feed additives.
- deletion of the existing possibility to dilute contaminated feed materials instead of decontamination or destruction (introduction of the principle of non-dilution).
- deletion of the possibility for derogation of the maximum limits for particular local reasons.
- introduction of the possibility of the establishment of an action threshold triggering an investigation to identify the source of contamination (“early warning system”) and to take measures to reduce or eliminate the contamination (“pro-active approach”).

In particular the introduction of the principle of non-dilution is an important and far-reaching measure. In order to protect public and animal health, it is important that the overall contamination of the food and feed chain is reduced to a level as low as reasonably achievable providing a high level of public health and animal health protection. The deletion of the possibility of dilution is a powerful means to stimulate all operators throughout the chain to apply the necessary preventive measures to avoid contamination as much as possible. The prohibition of dilution accompanied with the necessary control measures will effectively contribute to safer feed.

During the discussions in view of the adoption of Directive 2002/32/EC the Commission made the commitment to review the provisions laid down in Annex I on the basis of updated scientific risk assessments and taking into account the prohibition of any dilution of contaminated non-complying products intended for animal feed. The Commission has therefore requested the Scientific Committee on Animal Nutrition (SCAN) in March 2001 to provide these updated scientific risk assessments in order to enable the Commission to finalise this review as soon as possible (Question 121 on undesirable substances in feed)³.

It is worthwhile to note that Council Directive 1999/29/EC is a legal consolidation of Council Directive 74/63/EEC of 17 December 1973 on the undesirable substances in animal nutrition⁴, which has been frequently and substantially amended. Consequently, several of the provisions of the Annex to Directive 2002/32/EC date back from 1973.

¹ OJ L140, 30.5.2002, p. 10

² OJ L 115, 4.5.1999, p. 32

³ Summary record of the 135th SCAN Plenary meeting, Brussels, 21-22 March 2001, point 8 – New questions (http://europa.eu/comm/food/fs/sc/scan/out61_en.pdf)

⁴ OJ L 38, 11.2.1974, p. 31

The opinion on undesirable substances in feed, adopted by SCAN on 20 February 2003 and updated on 25 April 2003⁵ provides a comprehensive overview on the possible risks for animal and public health as a consequence of the presence of undesirable substances in animal feed.

On the basis of this opinion, some provisional amendments are proposed to the Annex of Directive 2002/32/EC in order to guarantee the supply of some essential, valuable feed materials as the level of an undesirable substance in some feed materials, due to normal background contamination, is in the range of or exceeds the maximum level laid down in Annex I of Directive 2002/32/EC. Also some inconsistencies in the provisions of the Annex have been observed.

It was nevertheless acknowledged by SCAN itself for several undesirable substances and by the Standing Committee on the Food Chain and Animal Health that additional detailed risk assessments are necessary to enable a complete review of the provisions in the Annex.

4. Specific Background

The most important sources of contamination are *Fusarium graminearum* and *Fusarium culmorum*, two typical field fungi. These species commonly contaminate cereal crops in Europe.

No maximum levels for deoxynivalenol (DON) in animal feed have been established in EU legislation. Several Member States have established national orientation/guideline values for the presence of DON in feed materials and feedingstuffs⁶.

Maximum levels for DON foodstuffs are currently under discussion at EU level.

SCAN concluded⁷ that DON is found in the majority of European cereal crops destined for animal feed and that chronic exposure of susceptible livestock (particularly pigs) can lead to problems of animal health and is a cause of significant economic loss.

TERMS OF REFERENCE

The European Commission requests the EFSA to provide a detailed scientific opinion on the presence of deoxynivalenol (DON) in animal feed.

This detailed scientific opinion should comprise:

- the determination of the toxic exposure levels (daily exposure) of DON for the different animal species of relevance (difference in sensitivity between animal species) above which
 - signs of toxicity can be observed (animal health/impact on animal health) or
- the level of transfer/carry-over of DON from the feed to the products of animal origin results in unacceptable levels of DON or of its metabolites in the products of animal origin in view of providing a high level of public health protection:

⁵ Opinion of the Scientific Committee on Animal Nutrition on Undesirable Substances in Feed, adopted on 20 February 2003, updated on 25 April 2003 (http://europa.eu/comm/food/fs/sc/scan/out126_bis_en.pdf)

⁶ Germany, Austria, Belgium, Sweden, Netherlands : orientation/guideline values established ranging from 400 µg/kg for feedingstuffs for pigs up to 5000 µg/kg for cattle and fattening poultry.

⁷ Opinion of the Scientific Committee on Animal Nutrition on Undesirable Substances in Feed, point 7.6. Conclusions and recommendations.

- the identification of feed materials which could be considered as sources of contamination by DON and the characterisation, insofar as possible, of the distribution of levels of contamination
- the assessment of the contribution of the different identified feed materials as sources of contamination by DON
 - to the overall exposure of the different relevant animal species to DON,
 - to the impact on animal health
 - to the contamination of food of animal origin (the impact on public health), taking into account dietary variations and carry-over rates⁸
- the identification of eventual gaps in the available data which need to be filled in order to complete the evaluation.

ASSESSMENT

3. Introduction

Deoxynivalenol (DON) is a mycotoxin belonging to the trichothecenes, a family of closely related compounds, produced by several plant pathogenic fungi, of which the *Fusarium* family is the most important. Trichothecenes are a heterogeneous group of stable tetracyclic sesquiterpenes, sharing a 12-13-epoxy moiety. DON is allocated to the B group of trichothecenes, characterized by a carbonyl function at C-8. It is chemically described as 12,13-epoxy-3 α ,7 α ,15-trihydroxy-trichothec-9-en-8-one (C₁₅H₂₀O₆, MW: 296.32, CAS 51481-10-8). DON crystallizes as colourless needles, has a high temperature tolerance (stable at 120°C, moderately stable at 180°C) and is soluble in water and in some polar solvents, including aqueous methanol, acetonitril, and ethyl acetate. A trivial name of DON is "vomitoxin", a name derived from the acute symptoms this toxin exerts to pigs, when ingested. The chemical structure of DON is given in figure 1.

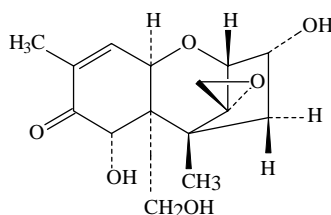


Figure 1. Chemical structure of deoxynivalenol

Two ubiquitous typical field fungi, *Fusarium graminearum* (*Gibberella zea*) and *Fusarium culmorum* are the most important sources of DON. The geographical distribution of the two species appears to be related to temperature, with *Fusarium graminearum* predominantly occurring in warmer climates. These fungi cause *Fusarium* head blight in wheat and *Gibberella* ear rot in maize. A direct relationship between the incidence of *Fusarium* head blight and

⁸ Importance of the human exposure to deoxynivalenol from foods of animal origin compared to overall human dietary deoxynivalenol exposure can be assessed making use of the information contained in the report on a task on human exposure assessment to deoxynivalenol which has been finalised in July 2003 at EU level within the framework of co-operation by Member States in the scientific examination of questions related to food (EC, 2003).

contamination of wheat with DON has been established. The incidence of *Fusarium* head blight is most affected by moisture at the time of flowering (WHO, 2001). The time of rainfall, rather than the amount, is the most critical factor for toxin production.

Among the trichothecenes DON is the most frequently occurring toxin, and is found worldwide, particularly in cereal crops such as wheat, maize, barley, oats, rye and less often in rice, sorghum and triticale. Under natural conditions, its two mono-acetylated derivatives 3- and 15-acetylDON accompany DON, albeit that these derivatives are produced at lower concentrations. Moreover, feeds may be co-contaminated with other mycotoxins produced by the same *Fusarium* species.

Compared to other trichothecenes, many data have been reported on the occurrence of DON in foods and feeds, and the EU-SCOOP report on *Fusarium* toxins has recently become available, which includes data on the occurrence of DON in food commodities from 11 European countries (EC, 2003).

Upon ingestion, DON is well absorbed and metabolised by de-epoxydation and glucuronidation, resulting in less toxic products. Elimination occurs by renal and biliary excretion, and only trace amounts are transferred into milk and eggs (D'Mello *et al.*, 1997, Jonker *et al.*, 1999).

The toxicity of DON is relatively well investigated, and the typical, dose-dependent toxic symptoms in laboratory animals as well as in farm animals comprise decreased feed intake, followed by reduction in weight gain, and at higher concentrations vomiting and feed refusal. (for review see Rotter *et al.*, 1996; Eriksen and Alexander, 1998, WHO, 2001).

Some cases of human food poisoning by contaminated grains have been reported. Symptoms described in human patients include abdominal pain or a feeling of fullness in the abdomen, dizziness, headache, throat irritation, nausea, vomiting, diarrhoea, and blood in the stool. These symptoms were readily reversible (Li *et al.*, 2002, Sudakin, 2003).

There is no experimental or epidemiological evidence for mutagenic and/or carcinogenic properties of DON and it was classified by the International Agency for Research on Cancer in Group 3 (not classifiable as to its carcinogenicity to humans) (IARC, 1993).

Risk assessments for DON have been performed by the Scientific Committee on Food (SCF, 2002), the Joint Expert Committee on Food Additives (JECFA) (WHO, 2001), and the Nordic Working Group (Eriksen and Alexander, 1998). A temporary tolerable daily intake (TDI) of 1 µg/kg body weight was established by the EU Scientific Committee on Food (SCF, 2002) that is in line with the temporary tolerable daily intake established by the Nordic Group (1998), and the provisional maximum tolerable daily intake (PMTDI) established by JECFA (WHO, 2001).

4. Analysis

For the monitoring of the occurrence of DON in food and feed commodities, various methods of analysis exist, reviewed by Langseth and Rundberget (1998), and Krska *et al.* (2001). Most methods for DON include solid phase extraction (SPE) cleanup, or immunoaffinity (IA) cleanup, in combination with chromatographic separation such as (capillary) gas chromatography with electron capture (GC-ECD), or mass spectroscopic detection (GC-MS detection), or liquid chromatography with ultraviolet (LC-UV), fluorescence (LC-FLD) or mass spectroscopic (LC-MS) detection. For screening purposes enzyme-linked immunosorbent assays (ELISA) can be used (Krska *et al.*, 2001). ELISAs are generally very sensitive, but at the same time, the uncertainty of the results is in general rather high. Despite these various analytical possibilities, formally (through collaborative studies) validated methods are scarce. AOAC validated methods exist for wheat (AOAC, 2000), but for (mixed) feedingstuffs no validated method is yet available. Recent FAPAS studies (Food Analysis Performance Assessment Scheme (FAPAS, 2000, 2002, 2003a,b) indicate that LC-UV is the preferred analytical technique, whereas GC-ECD, ELISA, GC-MS and LC-

MS are also used, but to a (much) lesser extent. These studies showed that satisfactory scores for the participants of the FAPAS study ranged from 60 % to 79 % for various wheat and maize test materials, contaminated at DON levels with assigned values ranging from 463 µg/kg to 2531 µg/kg. An EU analytical task force is currently studying the performance of a number of analytical methods for the determination of various trichothecenes (including DON) in food.

For inter-laboratory comparisons, certified reference materials are available. They consist of naturally contaminated wheat and maize flour and of blank materials of both matrices. The certified reference materials are available through the European Commission's Joint Research Centre/Institute for Reference Materials and Measurements (see <http://www.irmm.jrc.be>). A reference calibrant for DON is currently being developed in the frame of the FP5 project "DONCALIBRANT".

5. Current legislation

In 2003, approximately 40 countries around the world reported regulatory or guideline levels for DON in foods and animal feed (FAO, 2004). Whereas until 1996 DON was only sporadically regulated (FAO, 1997), it has become a toxin of high concern amongst regulatory authorities since the late 1990's, when mg/kg concentrations were reported to occur in cereals and cereal products. Several countries in Europe, Asia, North and Latin America have set limits for DON in wheat and other cereals, ranging from 300 – 2000 µg/kg. In many countries of the EU, a level of 750 µg/kg is set and applied as (unofficial) maximum limit for DON in flour used for human consumption since several years. Moreover, the European Commission issued a draft recommendation on DON in cereals and cereal products for human consumption in 2000 (EC, 2000) and the FAO/WHO *Codex Alimentarius* is also in the process of establishing maximum levels for DON in cereals and cereal products, including infant foods.

Worldwide 14 countries, including 6 individual EU Member States (Austria, Cyprus, Estonia, Lithuania, The Netherlands, Slovenia), have proposed also maximum limits for DON in various feedstuffs (including feeds for cattle, pigs, and poultry) (FAO, 2004). However, specific EU-harmonised limits have not been established yet.

6. Occurrence of deoxynivalenol in feed materials

DON occurrence is almost exclusively associated with cereals, and the levels of occurrence are in the order of hundreds of µg/kg upwards. DON occurs as a field (pre-harvest) rather than a storage contaminant, and almost always co-occurs with other *Fusarium* toxins. Preventive measures are difficult to implement, and even the effect of fungicide treatment on DON levels is controversial (Edwards *et al.*, 2001). As seasonal variations significantly influence the extent of *Fusarium* infections, levels of DON tend to vary from year-to-year making it difficult to generalise as to typical levels of occurrence.

A recent review of worldwide contamination of cereal grains and animal feed with *Fusarium* mycotoxins (Placinta *et al.*, 1999) has tabulated the finding of DON in wheat, maize, oats, barley, rye and feeds in Bulgaria, Finland, Germany, The Netherlands, Norway and Poland at levels ranging from a few µg/kg to more than 30 mg/kg. The highest reported levels were in maize cobs from Poland. In most cases nivalenol and zearalenone were found to co-occur with DON. Extensive compilation of data for DON in European cereals can be found in both, the JECFA safety evaluation of DON (WHO, 2001), and the SCOOP Report on *Fusarium* toxins (EC, 2003). Summaries of these data are tabulated in Table 1 and 2 of the Annex. The data from the reports are only partly based on the same analytical results. The JECFA report includes data mostly from the years 1986 to 1998, and the SCOOP report from 1996 to 2002. As both reports

were primarily focussed on assessing human exposure to DON and other toxins, inevitably the analysis centred on grain and grain products presumably destined for human food. These tables take only data on unprocessed grains, and there is the presumption that none of these samples were intended for animal feed (as the destination remained unspecified). Therefore, this data seems to represent a 'best case' situation. If surveillance were to be focussed on poorer quality grain that is frequently diverted to animal feed, a higher incidence and higher levels of DON would undoubtedly be found.

This overview (see table 1 of annex) indicates also a consistent picture of relatively low incidence of DON in oats, rye and barley, but frequent and sometimes very high levels of DON in samples of maize and wheat. Of particular note are the 115 samples of maize from France (44 % incidence) and 99 samples of maize from The Netherlands (72 % incidence) with DON levels above 500 µg/kg from 2001 surveys, and 444 samples of wheat (17 % incidence) from The Netherlands with similar contamination levels.

It is difficult to make direct comparisons between these two tables (Tables 1 and 2) as the results are reported in different formats. However, the results in table 2 show barley and maize to be the cereals with high incidence, highest mean and highest maximum values for DON. Wheat samples had a lower weighted mean DON level, but the very high number of samples included in the survey may skew these results, as incidentally very high levels of DON have been found in wheat. Survey work carried out in Hungary has been specifically targeted towards cereals intended for animal feed use (Rafai *et al.*, 2000) and between 1991 and 1998 analysis of maize, wheat, barley, oats, triticale, rye, bran, soybean and sunflower was carried out (1666 samples in total). These data cannot be compared directly with the SCOOP results, as in this case ranges of levels found and % of incidence are reported, but no detailed numbers of samples within specified ranges of DON contamination are provided. For maize, wheat and barley the ranges of DON contamination were reported to be 50 - 870, 70 - 1560 and 50 - 1200 µg/kg respectively. This paper (Rafai *et al.*, 2000) also reports DON in bran (50 - 960 µg/kg), soybean (60 - 720 µg/kg) and sunflower (150 µg/kg) intended for animal feed. Mycotoxin surveillance in the UK (Scudamore *et al.*, 1998) focussed on maize and maize products as ingredients of animal feed. Of 39 samples of maize gluten, 8 samples (20 %) contained DON levels above 500 µg/kg with the highest level being 5000 µg/kg. Of 24 samples of other maize products, 11 samples (45 %) contained DON levels above 500 µg/kg with the highest level of 4900 µg/kg being found. In a more recent UK study (Macdonald *et al.*, 2004) of 195 samples of wheat, barley and oats intended for animal feed, only 20 % of samples contained DON above 100 µg/kg and only one sample contained more than 500 µg/kg.

Studies on the milling of DON contaminated wheat and maize did show that DON is concentrated in by-products, the latter being often intended as animal feed. DON was found to be concentrated by a factor of 2 - 5 in wheat bran and with a factor 2 in wheat feed meal (Scott *et al.*, 1983, 1985; Hart and Braselton, 1983; Young *et al.*, 1984; Seitz *et al.*, 1985, 1986; Lee *et al.*, 1987). After dry milling of maize most of DON ends up in the maize germ meal. In contrast, wet milling gives DON concentrations similar to those in the original maize in the gluten and germ fractions, and several times higher in feed fraction including fibre and corn steep liquor (Patey and Gilbert, 1989; Lauren and Ringrose, 1997). DON is also accumulated through husk, small and broken kernels in cereal grain screenings, which is sometimes used as feedstuffs.

Even though it is apparent that co-occurrence of DON together with zearalenone and other Fusarium toxins (including nivalenol, 3-acetyl DON, fusarenone-X, moniliformin, and fumonisins) is common, it is not always easy to assess how many different toxins are present, and at what levels they were found in specific samples (Scudamore *et al.*, 1998; Müller and Schwadorf 1993).

Corn cob mix and maize silage have often found to be contaminated with DON in field cases and directed studies (Drochner *et al.*, 1984; Oldenburg *et al.*, 1996; Dieber and Kofer, 1999; Hochsteiner and Schuh, 2000, 2001). These feedingstuffs must be considered as important

sources of animal exposure, although no regular surveys have been published yet. Oldenburg and Höppner (2003) have published a survey of DON in forage maize from 2000 in Germany. A majority (59 %) of the 196 samples contained more than 300 µg/kg DON, and 9.2 % and 2.6 % contained more than 2,000 µg/kg and 5,000 µg/kg, respectively.

Recently, field cases in both the Netherlands and Sweden have shown the presence of high DON concentrations (in some cases together with zearalenone) in straw bedding for sows (5 wheat and 3 barley straw samples) (unpublished data of the Dutch Veterinary Field Service, Deventer, The Netherlands, and the Swedish Veterinary Institute and the Swedish Farmers' Selling and Purchasing Association, unpublished). In these straw samples concentrations up to 6,600 ppb have been detected. Previously, only moderate concentrations of DON in straw were reported (Benham, 1981, Schwadorf and Müller, 1995, Hörberg, 2001). As sows (and also horses) may consume a large portion of their straw bedding, this possible route of exposure needs to be taken into account at the farm level.

7. Estimating deoxynivalenol exposure of farm livestock

A quantitative estimate of the exposure of farm animals is impossible at present, as data on the occurrence of DON in feedstuffs is incomplete, as mentioned above.

Pigs and poultry are fed compounded diets, which are manufactured commercially or at the farm level. A standard diet consist of 40 - 80 % cereal grains, 0 - 50 % protein concentrate, 0 - 10 % oil and 0 - 10 % minerals, vitamins and other supplements. In general, the relative amount of protein concentrate is higher (and subsequently the cereal quantity lower), in diets for growing animals as compared to adults, and in diets for poultry higher than in those for pigs. Feedstuffs with high fibre contents (roughage-like feedstuffs) are only rarely used in diets for pigs and poultry. However, straw from bedding might be taken up by the animals (particularly sows) and might be an unexpected source of exposure.

The cereal proportion of the diets can principally contain all types of cereal grains that are cultivated in Europe but maize, wheat and barley and their (by)products are the most frequently used grains. Soybean meal is regularly used as protein concentrate, but in limited quantities for pigs. Other European protein sources include rape-products, sunflower meal, peas, field beans and lupines which are all used to a lesser extend, but might be of local importance in some regions.

The concentrate portion of ruminant diets is principally composed of the same feedstuffs as for monogastric species, however, the fraction of the individual components might be different from those normally used in diet formulation for pigs since nutritional requirements differ. The total amount of concentrate (cereal grains plus protein concentrates like soybeans) added to a ruminant diets, varies between 0 - 70 %, depending on the milk production level in diary cows and the farm management. Concentrate is given in addition to roughage such as fresh green fodder or conserved feedstuffs (silages, hay, straw).

Cereals (and other feeds) may contain DON and either of the two mono-acetylated derivatives 3- and 15-acetylDON. As the acetylated forms of DON are rapidly de-acetylated *in vivo*, no differentiation is made in most cases.

8. Adverse effects of DON

6.1. Mode of action

The first toxic effect associated with trichothecenes including DON was the inhibition of protein synthesis, the potency depending on structural constituents and requiring an unsaturated bond at the C9-C10 position and integrity of the 12, 13-epoxy ring. Trichothecenes bind to the 60S subunit of eukaryotic ribosomes and interfere with the activity of peptidyltransferase. Deoxynivalenol, which lacks a substituent at C-4, inhibits chain elongation (Ehrlich and Daigle, 1987). *In vitro*, deoxynivalenol is about 100 times less toxic than T-2 toxin with respect to protein inhibition (Ueno *et al.*, 1973, Thompson and Wannemacher, 1986).

Based on the induction of emesis (hence the name vomitoxin was coined), investigations towards the mechanisms involved in this effect pointed towards a possible interaction with serotonergic and dopaminergic receptors in the area postrema (Fioramonti *et al.*, 1993). Subsequently, loss of appetite, reduced feed intake resulting in delayed weight gain in growing animals (pigs) was attributed to this effect. In addition, early studies also described immunosuppression (allocated to a reduced production of immunoglobulins and depletion of lymphocytes from spleen, Peyer's patches and thymus).

More recently, evidence is accumulating that the principal effects of DON are related to an up-regulation of pro-inflammatory cytokines. Model experiments have indicated that DON increases the binding activities of transcription factors such as AP-1, NF κ B and C/EBP (Wong *et al.*, 2002, Pestka *et al.*, 2003), followed by and increased synthesis of TNF α and IL-6 and an induction of COX-2 (Wong *et al.*, 1998, Moon and Pestka, 2002). Moreover, stabilization of TNF α and IL-6 mRNA could be demonstrated (Wong *et al.*, 2002). These findings correspond to *in vivo* studies in mice, in which the increase in TNF α , IL-1 β , IL-6 and IFN γ production in spleen and Peyer's Patches was observed (Azcona-Olivera *et al.*, 1995; Zhou *et al.*, 1997, 1998). Other studies demonstrated the increase in IL-2, IL-4, IL-5 and IL-6 mRNA and protein expression in T-lymphocytes (Ouyang *et al.*, 1996, Warner *et al.*, 1994). The role of DON as pro-inflammatory agent was further substantiated by the finding that it up-regulates MIP-2 (Chung *et al.*, 2003).

Up-regulation of proinflammatory cytokines occur at low concentrations, whereas acute exposure to higher concentration results in apoptosis of leukocytes (Pestka *et al.*, 1994; Shifrin and Anderson, 1999; Islam *et al.*, 2002). Clinical studies revealed cell depletion in thymus, spleen or Bursa fabricius in exposed animals, and *in vitro*, the sensitivity of B- and T- cells, isolated from spleen, thymus and Peyer's patches towards DON has been described in many studies.

In vivo apoptosis of cells in thymus, spleen, Peyer's patches, bone marrow and liver has been also demonstrated after administration of other trichothecenes (Poapolathep *et al.*, 2002, 2003, Shinozuka *et al.*, 1997a,b).

The convincing evidence in rodent species that DON increases the expression of pro-inflammatory cytokines, might provide the explanation for a various effects observed in pigs, including:

- feed refusal, as IL-6 is known to induce anorexia,
- inhibition of protein-synthesis and reduced weight gain,
- IL-6 dependant deregulation of IgA production (elevated levels of circulatory IgA might impair glomerular function, resulting in a renal wasting syndrome (Pestka and Zhou, 2000).

In addition, these finding may also explain the diversity in clinical responses to lower levels of DON in individual herds, as the response to DON is strongly influenced by concomitant factors

such as LPS (the lipopolysaccharide of Gram-negative bacteria) and viral infections, modulating the transcription of cytokines and chemokines.

It is worthwhile to mention that these mechanistic studies have been almost entirely conducted in mice, or rodent and human cell lines. However, the described mechanisms are highly preserved in mammals. The response in pigs may be even more pronounced due to the renowned susceptibility of pigs to bacterial and viral agents modulating cytokine response.

6.2. Toxic effects in pigs

Many feeding studies of the effects of DON on pigs have been published (for a summary see Table 3 of the Annex). In these studies, either crystalline DON or naturally or artificially infected cereals have been mixed with feed. The effects of pure DON added to feed and naturally contaminated feed containing similar levels of DON have been compared in at least four feeding studies with pigs. Naturally infected feed had a stronger effect on the feed intake and weight gain than pure toxin in all these studies comparing the difference sources of DON (Forsyth *et al.*, 1977; Foster *et al.*, 1986; Rotter *et al.*, 1994; Trenholm *et al.*, 1994). The difference remains to be explained, but proposed hypotheses include the presence of other toxins in the material, the presence of other compounds (as for example bacterial polysaccharides) affecting the toxicity of trichothecenes and inducing taste aversion (Rotter *et al.*, 1996).

When crystalline DON was applied, complete feed refusal was observed at levels of 12 mg DON per kg feed and vomiting at 20 mg/kg feed (Young *et al.*, 1983; Forsyth *et al.*, 1977). Feeding trials where naturally or artificially infected material has been mixed into the diet show decreased feed consumption and weight gain in pigs already at doses from 0.6 – 2 mg DON/kg feed (Friend *et al.*, 1982; Overnes *et al.*, 1997; Bergsjö *et al.*, 1993b; Young *et al.*, 1983). The consumption of DON-contaminated feed has been associated also with epithelial lesions in the oesophageal region of the stomach when pigs have been given naturally infected feed containing from about 3 - 6 mg DON/kg feed (Cote *et al.*, 1985; Foster *et al.*, 1986; Friend *et al.*, 1986b; Rotter *et al.*, 1992, 1995). The observed reduction in feed intake at the lowest doses was temporary, but the loss in weight gain during the first period was not completely compensated for during the later periods and the animals reach slaughter weight at a higher age.

The negative effect on feed intake in pigs is generally considered as most sensitive endpoint of toxicity. In many studies, particularly those conducted with purified toxin, no effect has been found at levels from 0.6 – 0.9 mg DON/kg feed, but in two studies conducted with naturally contaminated grains a temporary reduction on feed intake was observed even in pigs given 0.35 mg DON/kg feed (Friend *et al.*, 1982; Trenholm *et al.*, 1983) (for details see table 3).

Changes in different clinical chemistry parameters (plasma nutrients and plasma enzyme activities) have been reported from several studies (Bergsjö *et al.*, 1993b; Young *et al.*, 1983; Cote *et al.*, 1985; Chavez, 1984; Lusky *et al.*, 1998; Döll *et al.*, 2003) while in other experiments no changes were observed (Dänicke *et al.*, 2004e, g, c). Reported alterations are probably due to the reduced feed intake and not a direct effect of the toxin since no changes in these parameters were observed when compared to pair-fed controls (Lun *et al.*, 1985).

Changes in kidney tubular epithelium were observed in 1 of 6 pigs in the group given 1.0 mg DON/kg feed (toxin containing fungal culture material added to the diet) for 90 days while no such changes were found in the two control pigs (Lusky *et al.*, 1998).

A few studies of the effects of DON on pig reproduction have been published. Statistical significant trends towards a lower foetal weight and length were reported from sows fed 0.1, 1.7 or 3.5 mg DON/kg feed during pregnancy when the foetuses were examined 50 - 54 days of gestation (Friend *et al.*, 1983; Chavez, 1984). The foetal mortality rate was not significantly different from those among the groups. Moreover, no gross malformations were observed. In another study, Yorkshire gilts were fed restricted quantities of wheat diets, containing DON at a concentration of 0.2, 3.8 or 6.2 mg/kg (naturally contaminated) feed. No effects of DON on litter

size, weight or size at birth, or weight gain during lactation or survival rate of piglets have been found (Friend *et al.*, 1986a).

A few studies of the immune response towards vaccinations have been carried out. Rotter *et al.* (1994) found a delayed immune response towards sheep red blood cells (SRBC), one and two weeks after immunisation in pigs given 3 mg DON/kg feed. The response was significantly reduced compared to a pair-fed control group, but not from a control group fed *ad lib*. A significant dose-related reduction in antibody response towards tetanus toxoid was observed nine weeks after the start of the experiment in pigs fed 1.8 or 4.7 mg DON/kg feed in the diet compared to control pigs. No effect was found on the four other antigens tested, including SRBC, or in response towards any antigen after six weeks of exposure (Overnes *et al.*, 1997).

A statistically significant decreasing trend in serum α -globulin was found with increasing DON-levels in pigs given 0 - 3 mg DON/kg feed (Rotter *et al.*, 1994). Decreased plasma β -globulin concentrations were found in growing pigs fed 4.0 mg DON/kg feed after 2 and 3 weeks, but not after 6 weeks (Rotter *et al.*, 1995). Similarly, a reduction in α -globulins was found in pigs fed 3 mg DON/kg feed for 18 days, but not in pigs given 1 mg DON/kg feed (Prelusky *et al.*, 1994).

6.3. Toxic effects in cattle

With regard to cattle, only a few feeding studies with dairy cows are available. Cows have been given a concentrate containing 6.4 mg DON/kg feed for 6 - 10 weeks (Trenholm *et al.*, 1985) or complete diet containing 8.5 mg DON/kg for 3 weeks (Ingalls, 1996). A slight temporary decrease in consumption of the concentrate was recorded when the concentrate concentration of DON was increased from 1.5 to 6.4 mg /kg concentrate in the first study. No effect was seen on weight gain, or hay consumption. The concentrate consumption returned to the previous levels when the cows after 6 - 10 weeks again were fed the concentrate containing 1.5 mg DON/kg. In the second study, no significant effect was found on feed intake, weight gain, milk yield, milk composition and rumen pH. A non-significant tendency towards a reduced feed intake in the third week was observed, but according to the authors this decrease was not related to levels of DON. No sign of illness was observed in the studies (Trenholm *et al.*, 1985; Ingalls, 1996). The latter study gives no information about either analytical methods or uncertainties, making it difficult to assess the information from the study.

The pH-value and the concentration of volatile fatty acids in the rumen fluid were not significantly influenced by feeding contaminated wheat to dry cows at dietary DON concentrations of 3.1 and 3.5 mg/kg (88 % dry matter basis). In contrast, the postprandial ammonia concentration was consistently higher when the mycotoxin-contaminated wheat was fed. This might be the result of the higher protein intake due to the higher crude protein concentration of the contaminated wheat and/or due to a decreased capacity of rumen microbes to utilize the released ammonium for microbial protein synthesis (Dänicke *et al.*, 2004a).

The inclusion of 5.0 or 12.1 mg DON/kg dry matter in a feed concentrate for 10 weeks had no effect on feed intake or milk yield.

6.4. Toxic effects in sheep

Even if various studies of the kinetics of DON in sheep have been reported, very few feeding trials studying toxic effects of DON on sheep are available. Harvey *et al.* (1986) fed lambs with a wheat diet containing 15.6 mg DON/kg feed for 28 days. Feed consumption, weight gain, and feed efficiency of the lambs given DON did not differ from the control animals. No differences between treated and untreated lambs were noted for haematological or serum biochemical variables and no gross or microscopic lesions were observed in treated lambs. In another study, intraruminal dosing with 5 mg DON/kg b.w. resulted in a 44 % decline in feed intake and a decrease of 5 % in apparent digestibility of the feed in sheep (Brewer *et al.*, 1996). The dose

used in this experiment was very high and it is unlikely to occur under practical conditions. Feeding a diet containing approximately 4.6 mg of DON and 0.34 mg ZEA per kg of complete ration, at a reference dry matter content of 88 %, did not impair rumen fermentation (pH, concentration of short chain fatty acids and ammonia) although there was a trend for a decrease in the rate of ruminal dry matter degradation (*in sacco*) of the slowly degradable wheat straw (Dänicke, 2002a and b).

6.5. Toxic effects in poultry

Many studies of the effects on DON in poultry of different age groups have been published (for details see Table 4).

Chickens have obviously a low sensitivity towards DON (as compared to pigs), and feed refusal and reduced weight gain are only found when concentrations reach 16 - 20 mg DON/kg feed (Kubena *et al.*, 1987a; 1988; 1989; Kubena and Harvey, 1988; Harvey *et al.*, 1991). Kubena *et al.* (1985) describe, however, a decrease in the relative and absolute liver weight and an increase on the relative and absolute gizzard weight in chickens fed 9 or 18 mg DON/kg feed for days 1 - 35 of age. No changes in weight gain, feed intake or the relative organ weight of other organs were found.

In conclusions, as yet no obvious relation between dietary DON concentration and reduced feed intake and body weight gain has been reported. Breed and feeding have changed, however, considerable during the last few years and are not comparable with those at which most of the experiments are conducted, thus limiting data interpretation.

Serum chemical and hematological parameters did not respond in broiler chickens when the dietary DON concentration reached 14 mg/kg (Dänicke *et al.*, 2003). The weight of the spleen relative to the body weight was significantly decreased whereas that of heart increased with increasing dietary DON concentrations. Protein utilization and protein digestibility were significantly higher when the contaminated wheat was fed.

Harvey *et al.* (1991) conducted a series of experiments investigating the effects of DON on the different parameters of the immune system of growing chickens. A reduced response towards vaccination against Newcastle disease virus (NDV) was observed in female Leghorn chickens given a diet based on naturally infected wheat containing 18 mg DON /kg feed for 18 weeks. No effect was found in broilers given the same diet for 9 weeks only. The blastogenic response of splenocytes was significantly reduced in the female Leghorn chickens given the wheat diet containing 18 mg DON/kg feed. A comparable effect was not observed in males given the same diet, nor in male or female Leghorn chickens given a diet containing 50 mg crystalline DON/kg feed. The blastogenic response was however significantly reduced in female animals given 50 mg pure DON/kg feed. The findings suggest that Leghorn chicks are less sensitive than broiler chicks (Hubbard x Hubbard), and that male chickens are less sensitive than females. Moreover, it was confirmed that pure (crystalline) toxin is less toxic than naturally contaminated feed materials. Dänicke *et al.* (2003) found the antibody titers to NDV (New Castle Disease Virus) to be linearly decreased in broilers given a diet that contained DON (from naturally contaminated wheat) at a concentration of 7 and 14 mg/kg feed. Feeding of a diet containing 12 mg DON/kg from contaminated maize to laying hens resulted also in a significant decrease in the serum antibody titers to the NDV, whereas the antibody titers to the bacterial antigen K88 were increased in the yolks at the same time (Dänicke *et al.*, 2002c).

In laying hens fed contaminated maize containing 12 mg/kg DON, feed intake of hens was significantly depressed only at the beginning of the 16 weeks lasting experimental period, but reached the level of the control group in the course of the experiment (Dänicke *et al.*, 2002c). DON up to 83 mg/kg feed did not have a significant effect on the egg production in laying hens. Hatchability was not affected by levels of up to 18 mg DON/kg feed (Hamilton *et al.*, 1985; Kubena *et al.*, 1987b; Bergsjö *et al.*, 1993a; Lun *et al.*, 1986). However, a small increase in the

incidence of minor malformations, considered as delayed foetal maturation (delayed ossification, un-withdrawn yolk sac) were observed in chick foetuses from hens given a feed containing 2.5 or 3.1 mg DON/kg, but not in the group given 4.9 mg DON/kg feed (Bergsjö *et al.*, 1993a). In a previous study, in which hens were given 0 - 4.9 mg DON/kg feed (Hamilton *et al.*, 1985), no abnormalities in chickens were recorded.

In Peking ducks, no significant differences in feed intake, body weight gain, and feed to gain ratio was observed at DON concentrations up to 7 mg/kg feed, although during the 1st week of the experiment a slight depression in weight gain was observed, which was, however, fully compensated during within the experimental period of 7 weeks. Gross macroscopical inspection of the upper digestive tract did not reveal any signs of irritation, inflammation or other pathological changes. The relative organ weight of the bursa of *Fabricius* decreased dose dependently. Activities of glutamate-dehydrogenase and γ -glutamyl-transferase in serum were not or inconsistently affected by DON exposure.

Feeding contaminated wheat containing DON at concentrations of 3 - 4.7 mg/kg feed to Peking ducks prevented an increase of the viscosity of the intestinal digesta, which is often seen as undesirable effect of diets high in grains, pointing towards the enzymatic activities of the invading *Fusarium* strain (Dänicke *et al.*, 2004b; Matthäus *et al.*, 2004).

6.5. Toxic effects in horses

Barley contaminated with 36 - 44 ppm DON (equivalent to a daily dose of approximately 11 mg per animal) was fed to 5 healthy horses for 40 days (Johnson *et al.*, 1997). Blood for clinical investigations was sampled every 10th day. Haematocrit values decreased slightly in a linear fashion, but no change in the peripheral white blood cell counts, polymorphonuclear leukocytes, and lymphocyte counts were not detected. No changes in serum creatinine, sodium, potassium, chloride, total calcium, and inorganic phosphate were detected. Serum enzyme activities of GGT (gamma glutamyltransferase), AST (aspartate aminotransferase) and creatine kinase decreased slightly in a linear fashion during the experimental period. Total serum protein, serum albumin and globulin, as well as serum IgG and IgA decreased also. The changes in these biochemical parameters were, however, non-significant and all values remained within the normal range. The horses showed no feed refusal or any other signs of toxicity.

6.6. Toxic effects in rabbits

Very limited data are available on the potential adverse effects of DON on rabbits, but the lack of case reports, and the two available studies indicate a high tolerance of rabbits to DON, which is in contrast to T-2 toxin, the latter exerting severe toxic symptoms in rabbits (Khera *et al.*, 1986, Biro, 2003).

6.7. Toxic effects in dogs

Contaminated wheat, containing DON at a concentration of 37 mg/kg was mixed into the diet for Brittany and Beagle dogs. Two to 14 dogs per group were fed 0, 1, 2, 4, 6, 8 or 10 mg DON per kg extruded feed, respectively, for 14 days. Vomiting occurred in dogs fed either 8 or 10 mg DON per kg feed. Feed intake was reduced. When extrapolating a NOEL from the energy intake curve, a threshold value of 4.5 ± 1.7 mg DON per kg feed was calculated. Dogs were able to preferentially select uncontaminated feed in comparison to a diet containing 6 mg DON per kg feed (Hughes *et al.* 1999).

6.8. Toxic effects in cats

Cats (American shorthaired) were given graded amounts of DON, from naturally contaminated wheat, with their diets during a period of 14 days (Hughes *et al.* 1999). Two cats per group were used for the levels 0, 1, 2, 4, 6 and 8 mg DON per kg feed, respectively, and 8 cats in the group 10 mg. Reduced feed intake and vomiting were observed only in the group with the highest DON concentration.

9. Toxicokinetics

7.1. Toxicokinetics in pigs

DON is rapidly absorbed in pigs and oral bioavailability is estimated to reach 55 %, whereas in ruminants only 2 – 3 % of the given dose is absorbed (Rotter, 1996). After intragastric dosing of radiolabeled DON, absorption half time was less than 30 minutes (Prelusky *et al.*, 1988). After feeding a diet containing naturally contaminated wheat (4.2 mg/kg feed), the maximum serum DON concentration was found after 4.1 h (Dänicke *et al.*, 2004d). The absorption half time in pigs fed 2.5 ppm 3-acetyl-DON has been estimated to be 1.26 hours (Eriksen *et al.*, 2003).

Organ distribution was measured in pigs only following a single intravenous injection of DON (1 mg/kg b.w.) and revealed high initial concentrations in plasma, kidney and liver. Measurable concentrations were, however, detected also in the abdominal fat, back fat, lung, adrenals, spleen, testis, heart, brain, muscle tissue, intestines and pancreas, indicating a large volume of distribution (Prelusky and Trenholm, 1991).

The plasma elimination half-life was found to vary between 1.2 and 3.9 hours in pigs depending on study (Eriksen *et al.* 2003; Prelusky and Trenholm, 1991; Coppock *et al.*, 1985). When radio-labelled DON was given by gavage, plasma clearance was found to be 7.14 hours (Prelusky *et al.*, 1988).

Excretion of DON occurs predominantly via urine, and in pigs given radiolabelled DON at a dose of 0.6 mg/kg b.w. intragastrically, or 0.3 mg/kg b.w. intravenously, 93 % of the administered dose was detected in urine. After oral dosing, 2.5 % of the dose was found to be excreted with bile 20 % with faeces and 68 % with urine (Prelusky *et al.*, 1988).

DON may be de-epoxidated by the microbial flora of the intestinal tract with an increasing capacity from the small to the large intestine (Dänicke *et al.*, 2004d). De-epoxy DON has not been detected in blood, although it was excreted in the urine (Eriksen *et al.*, 2003; Dänicke *et al.*, 2004e-g). Moreover, the glucuronidated DON is found in blood and urine, as well. Acetyl-DON is rapidly deacetylated in the upper intestinal tract and absorbed exclusively as de-acetylated DON (Eriksen *et al.*, 2003).

7.2. Toxicokinetics in cattle

DON is metabolised by the ruminal flora to the less toxic metabolite de-epoxy DON (Swanson *et al.*, 1987). Acetyl-DON is rapidly de-acetylated in the rumen to DON and subsequently de-epoxidated. Thus, in ruminants only minor amounts of DON will reach systemic circulation. For example, a single oral dose of 920 mg DON to each of two dairy cows resulted in a serum levels of 200 ng/mL and 90 ng/mL, after 4.7 and 3.5 hours, respectively (Prelusky *et al.*, 1984). A high percentage (24 – 46 %) of the serum DON concentration represented the conjugated form. Only trace concentrations (< 2ng/mL) were found in plasma after 24 hours.

7.3. Toxicokinetics in sheep

The excretion of DON after intraruminal administration of 5.0 mg DON/kg body weight has been studied in two sheep (Prelusky *et al.*, 1986b). DON was mainly excreted with urine with an average elimination half-life of 3.2 hours. About 50 % of the excreted DON was conjugated to glucuronic acid. De-epoxy DON and its glucuronide accounted for 24 %. Radio-labelled DON (4 mg/kg b.w.) has also been administered intravenously to lactating ewes (Prelusky *et al.*, 1987b), and found to be eliminated rapidly from plasma with a half-life of 1.1 hour. About 0.25 % of the dose was excreted into milk and mainly as de-epoxy DON, both conjugated and free (see below).

7.4. Toxicokinetics in poultry

The disposition of radio-labeled ¹⁴C-DON given at a singled dose (2.2 mg per animal) was studied in hens. DON was found to be poorly absorbed, as peak plasma levels at 2 - 2.5 hours accounted for less than 1 % of the administered dose. Maximum tissue levels were measured at 3 hours in liver, kidney, heart, spleen and gizzard, while for muscle and fat the maximum radioactivity was measured after 6 hours. Clearance of radioactivity from tissue had an average half-life of 16.83 ± 8.2 hours (range 7.7 - 33.3 hr depending on tissue). Elimination of the radio-labeled toxin into excreta occurred rapidly and recovered radioactivity accounted for 78.6, 92.1 and 98.5 % of the dose after 24, 48 and 72 hours, respectively (Prelusky *et al.*, 1986a).

Concentrations of DON and its de-epoxidized metabolite in plasma and bile of Peking ducks exposed to diets containing up to 6 - 7 mg DON/kg diet were lower than the detection limits of 6 ng/mL and 16 ng/mL, respectively, of the applied HPLC-method (Dänicke *et al.*, 2004c).

10. Carry-over and residues

Residue analyses of DON in tissues from pigs fed DON concentration ranging between 0.7 - 7.6 ppm DON revealed in most cases low (< 20 ppb) or undetectable DON concentrations (Pollman *et al.*, 1985; Cote *et al.*, 1985; Prelusky and Trenholm, 1992). Variable results are summarized in Table 5. Residues of DON in tissues from dairy cows have not been determined.

In poultry, residues of DON (detection limit 10 ng/g), have neither been found in tissues from chickens fed either 4 ppm for 28 days, 9 or 18 ppm for 35 days, and 83 ppm for 27 days, nor in eggs from laying hens fed 5 ppm for 190 days, 18 ppm for 28 days, and 83 ppm for 27 days, respectively (El-Banna *et al.*, 1983; Kubena *et al.*, 1985, 1987a; Lun *et al.*, 1986). An residual amount of 20 ng/g was, however found in the gizzard from laying hens fed 83 mg DON per kg feed for 182 days (Lun *et al.*, 1986). The chemical nature of the residue remained unknown.

Transmission of DON into eggs is limited (for details see table 5). Following a single oral administration of ¹⁴C-DON (2.2 mg) to laying hens, only 0.087 % (equivalent to 32 ppb) of the given doses was detected in the first egg (Prelusky *et al.*, 1987a). When feeding was continued with 20 ppm radio-labelled DON for six days, the levels increased with each subsequent egg. Maximum levels were equivalent to 70 ng/g of DON and its metabolites in the eggs.

In another study with 5.5 mg radio-labelled DON/kg fed to six laying hens for 65 days (Prelusky *et al.*, 1989), radioactivity in eggs increased to a maximum level of 1.7 µg DON equiv./egg at the 8th day of exposure. Thereafter radioactivity in the eggs decreased to about 25 % of this initial maximum value during the feeding period of 30 days, and remained relatively constant for the rest of the exposure period (in total 65 days). Thereafter, radioactivity in the eggs quickly dropped to negligible values when the exposure to DON ceased (Prelusky *et al.*, 1989).

The carry-over of DON into milk has been investigated in different studies (for details see table 5). Neither DON nor its de-epoxide metabolite or conjugates were detected in dairy milk at a detection limit of 1 ng/mL following the application of a single bolus of 1.7 mg/kg b.w.

(Charmley *et al.*, 1993). Following a single oral dose of 920 mg DON/animal (Prelusky *et al.*, 1984) or a dietary concentration of 66 mg DON/kg feed concentrate for 5 days (Cote, *et al.*, 1986), no measurable amounts of DON were found in the milk, but the de-epoxide metabolite was found to be excreted in milk over a period of 5 days in one individual dairy cow, given approximately 300 mg DON/day in the feed for 5 days (Cote *et al.*, 1986).

In ewes given radio labelled DON (4 mg/kg b.w.) approximately 0.25 % of the dose was excreted with milk, mainly as de-epoxy DON, both conjugated and free. Transmission of DON and metabolites into the milk from sheep fed a DON-contaminated (880 mg/kg diet) resulted in milk levels of 110 - 200 ng/mL.

11. Human dietary exposure

Human exposure to DON occurs predominantly via ingestion of cereals and grains, and therefore animal products do not significantly contribute to this exposure. Considering the year-to-year variability of the contamination of cereals and grains with DON in Europe, an exposure of consumers close to the TDI of 1 µg/kg b.w./day is possible, as concluded by the SCF (1999 and 2002) and confirmed by the recent SCOOP task (EC, 2003).

Dietary exposure varies considerably according to traditional food supplies in different geographic regions. The WHO presented a comparative estimate of human exposure in Europe, the Far East, Latin America and the Middle East. This comparison demonstrated that in Europe, approximately 80 % of the total DON intake is associated with the consumption of wheat, whereas in the Far East, wheat and rice are equally important as source of DON exposure (WHO, 2001).

CONCLUSIONS

- The mycotoxin deoxynivalenol (DON, vomitoxin), produced by different *Fusarium* species, is a frequent contaminant of various cereals and grains, particularly wheat, barley and maize, and their by-products. Mould invasion and subsequent toxin production occurs predominantly at the pre-harvest stage, and cannot readily be avoided under the conditions of current agricultural practice.
- The actual toxin concentration in feed materials varies considerably depending on climatic, seasonal and geographic conditions, as well as on genetic predisposition of individual hosting crops, and on the applied agricultural practice. Concentrations ranging from several micrograms up to several milligrams per kilogram have been found in feedingstuffs.
- In naturally contaminated feed materials DON is normally present together with its two mono-acetylated derivatives 3- and 15-acetylDON. At the same time, feeds may be co-contaminated with other mycotoxins produced by the same *Fusarium* species.
- A quantitative exposure assessment of target animal species is not possible, as no comprehensive surveys of DON concentrations in different feed materials are available.

- DON exhibits toxic effects in all animal species so far investigated, as well as in humans. Susceptibility varies considerably amongst species, but pigs are generally recognised as the most sensitive animal species.
- The initial adverse effect observed after DON exposure is reduced feed intake. At higher toxin concentrations vomiting and feed refusal will occur. These effects lead to a reduced body weight gain, particularly in growing animals. DON affects the immune response, and the release of pro-inflammatory cytokines is one of the earliest manifestations of exposure. At present, the available data do not allow the establishment of a no-effect level for pigs; the lowest reported levels with a negative effect on feed intake vary between 0.35 and 0.9 mg/kg feed.
- With respect to other animal species, it seems that healthy ruminants tolerate several milligram of DON/kg dry matter in the diet, due to the degrading capacity of the rumen flora. Poultry are also less sensitive than pigs to the effects of DON on feed intake and weight gain, but available data do not allow estimating a maximum tolerance level. Other species, including rabbits, horses, cats and dogs, seem to have a higher tolerance towards DON than pigs.
- DON is rapidly metabolised in the animal organism and the carry-over into edible tissues, milk and eggs is very low. Thus, animal derived foods contribute only marginally to total human exposure to DON.

RECOMMENDATIONS

- More data on occurrence of DON in feed materials and bedding (as opposed to cereals intended for human consumption) are needed to improve exposure assessment of animals.
- Analytical methods of appropriate sensitivity for feedingstuffs need to be validated by collaborative trials.
- For pigs, poultry and other relevant species, the no-effect level for reduced feed intake (and other adverse effects) needs to be refined or established, taking into account also the immunomodulatory effects of DON.

DOCUMENTATION PROVIDED TO EFSA

EC (European Community), 2003. Final report from SCOOP task 3.2.10: Collection of Occurrence Data of *Fusarium* Toxins in Food and Assessment of Dietary Intake by the Population of EU Member States, April 2003.

REFERENCES

- AOAC® (Association of Analytical Communities) 2000. Official Methods of Analysis. Deoxynivalenol in Wheat, Thin Layer Chromatography Method. Sec. 986.17, pp. 1205-1206. AOAC International.
- Azcona-Olivera, J.I., Ouyang, Y., Murtha, J., Chu, F.S., Pestka, J.J. 1995a. Induction of cytokine mRNAs in mice after oral exposure to the trichothecene vomitoxin (deoxynivalenol): relationship to toxin distribution and protein synthesis inhibition. *Toxicol. Appl. Pharmacol.* **133**, 109-120.
- Benham, C L. 1981. Moulds and mycotoxins in animal feedstuffs 1980. Proceeding of a fourth meeting on mycotoxins in animal disease. MAFF Reference book 360, 38-40.
- Bergsjö, B., Matre, T. and Nafstad, I. 1992. Effects of diets with graded levels of deoxynivalenol on performance in growing pigs. *Zentralbl. Veterinarmed. [A]* **39**, 752-758.
- Bergsjö, B., Herstad, O. and Nafstad, I. 1993a. Effects of feeding deoxynivalenol-contaminated oats on reproduction performance in White Leghorn hens. *Br. Poult. Sci.* **34**, 147-159.
- Bergsjö, B., Langseth, W., Nafstad, I., Jansen, J.H. and Larsen, H.J. 1993b. The effects of naturally deoxynivalenol-contaminated oats on the clinical condition, blood parameters, performance and carcass composition of growing pigs. *Vet. Res. Commun.* **17**, 283-294.
- Bergsjö, B. and Kaldhusdal, M., 1994. No association found between the ascites syndrome in broilers and feeding of oats contaminated with deoxynivalenol up to thirty-five days of age. *Poult. Sci.* **73**, 1758-1762.
- Biro, K. 2003. Adverse effects of deoxynivalenol and ochratoxin A in farm animals: comparative in vivo and in vitro studies. Thesis Utrecht University.
- Brewer, D., McAlees, A.J. and Taylor, A. 1996. Ovine ill-thrift in Nova Scotia. **13**. Anorexia and digestibility decline in female lambs given 3,7,11-3H3-3-acetoxy-7,15-dihydroxy-12,3-epoxytrichothec-9-en-8-one. *Proc. Nova Scotia Inst. Sci.* **41**, 39-47.
- Charmley, E., Trenholm, H.L., Thompson, B.K., Vudathala, D., Nicholson, J.W.G., Prelusky, D.B. and Charmley, L.L. 1993. Influence of Level of Deoxynivalenol in the Diet of Dairy Cows on Feed Intake, Milk Production, and Its Composition. *J. Dairy Sci.* **76**, 3580-3587.
- Chavez, E.R. 1984. Vomitoxin-contaminated wheat in pig diets: regnant and lactating gilts and weaners. *Can. J. Anim. Sci.* **64**, 717-723.
- Chavez, E.R. and Rheaume, J.A. 1986. The significance of the reduced feed consumption observed in growing pigs fed vomitoxin-contaminated diets. *Can. J. Anim. Sci.* **66**, 277-287.
- Chung Y.J., Yang, G., Islam, Z., Pestka, J.J. 2003. Up-regulation of macrophage inflammatory protein-2 and complement 3a receptor by the trichothecenes deoxynivalenol and satratoxin G. *Toxicology* **186**, 51-65.
- Coppock, R.W., Swanson, S.P., Gelberg, H.B., Koritz, G.D., Hoffman, W.E., Buck, W.B. and Vesonder, R.F. 1985. Preliminary study of the pharmacokinetics and toxicopathy of deoxynivalenol (vomitoxin) in swine. *Am. J. Vet. Res.* **46**, 169-174.
- Cote, L.M., Beasley, V.R., Bratich, P.M., Swanson, S.P., Shivaprasad, H.L. and Buck, W.B. 1985. Sex-related reduced weight gains in growing swine fed diets containing deoxynivalenol. *J. Anim. Sci.* **61**, 942-950.
- Cote, L.M., Dahlem, A.M., Yoshizawa, T., Swanson, S.P. and Buck, W.B. 1986. Excretion of deoxynivalenol and its metabolite in milk, urine, and feces of lactating dairy-cows. *J. Dairy Sci.* **69**, 2416-2423.

- D'Mello JPF, Porter JK, MacDonald AMC, Placinta CM (1997). Fusarium mycotoxins. In: D'Mello JPF (ed). Handbook of Plant and Fungal Toxicants, Boca Raton, Florida: CRC press, p. 287.
- Dänicke, S., 2002a. Effects of Fusarium toxin contaminated wheat grain and of a detoxifying agent on rumen physiological parameters and in sacco dry matter degradation of wheat straw and lucerne hay in wethers. *J. Anim. Feed Sci.* **11**, 437-451.
- Dänicke, S., Gädeken, D., Ueberschär, K.H., Meyer, U., Scholz, H., 2002b. Effects of fusarium toxin contaminated wheat and of a detoxifying agent on performance of growing bulls, on nutrient digestibility in wethers and on the carry-over of zearalenone. *Arch. Anim. Nutr.* **56**, 245-261.
- Dänicke, S., Ueberschär, K.H., Halle, I., Matthes, S., Valenta, H., Flachowsky, G., 2002c. Effect of addition of a detoxifying agent to laying hen diets containing uncontaminated or Fusarium toxin-contaminated maize on performance of hens and on carryover of zearalenone. *Poultry Sci.* **81**, 1671-1680.
- Dänicke, S., Matthes, S., Halle, I., Ueberschär, K.H., Döll, S. and Valenta, H. 2003. Effects of graded levels of Fusarium toxin-contaminated wheat and of a detoxifying agent in broiler diets on performance, nutrient digestibility and blood chemical parameters. *Brit. Poultry Science.* **44**, 113-126.
- Dänicke, S., Matthäus, K., Lebzien, P., Valenta, H., Stemme, K., Ueberschär, K.-H., Flachowsky, G., 2004a. On the effects of Fusarium toxin contaminated wheat grain on the fermentation and utilization of nutrients in the rumen and on ruminal metabolism of deoxynivalenol and zearalenone of dairy cows. *J. Anim. Physiol. An. N.* Accepted,
- Dänicke, S., Matthäus, K., Valenta, H., Halle, I., 2004b. Effects of Fusarium-toxin contaminated wheat grains and non-starch-polysaccharide (NSP) hydrolyzing enzyme preparation on Pekin duck performance. *Archiv für Geflügelkunde.* In press,
- Dänicke, S., Ueberschär, K.-H., Valenta, H., Matthes, S., Matthäus, K., Halle, I., 2004c. Effects of graded levels of Fusarium toxin-contaminated wheat in Pekin duck diets on performance, health and metabolism of deoxynivalenol and zearalenone. *Brit. Poultry Sci.* In press,
- Dänicke, S., Valenta, H., Döll, S., 2004d. On the toxicokinetics and the metabolism of deoxynivalenol (DON) in the pig. *Arch. Anim. Nutr.* **58**, 169-180.
- Dänicke, S., Valenta, H., Döll, S., Ganter, M., Flachowsky, G., 2004e. On the effectiveness of a detoxifying agent in preventing fusario-toxicosis in fattening pigs. *Anim. Feed Sci. Tech.* In press,
- Dänicke, S., Valenta, H., Goyarts, T., Razzazi, E., Böhm, J., 2004f. On the effects of increasing deoxynivalenol (DON) concentrations in pig feed on growth performance and utilization of nutrients and on DON metabolism. *J. Anim. Feed Sci.* Accepted,
- Dänicke, S., Valenta, H., Klobasa, F., Döll, S., Ganter, M., Flachowsky, G., 2004g. Effects of graded levels of Fusarium toxin contaminated wheat in diets for fattening pigs on growth performance, nutrient digestibility, deoxynivalenol balance and clinical serum characteristics. *Arch. Anim. Nutr.* **58**, 1-17.
- Dieber, F. and Kofer, J. 1999. Mykotoxinbelastung von steirischem Mais - Abhängigkeit von Menge und zeitlicher Verteilung der Niederschläge. *Ernährung* **23**, 294-298.
- Döll, S., Dänicke, S., Ueberschär, K.H., Valenta, H., Schnurrbusch, U., Ganter, M., Klobasa, F. and Flachowsky, G. 2003. Effects of graded levels of Fusarium toxin contaminated maize in diets for female weaned piglets. *Arch. Tierernähr.* **57**, 311-334.
- Döll, S., Gericke, S., Dänicke, S., Raila, J., Ueberschär, K.-H., Valenta, H., Schnurrbusch, U., Schweigert, F. J., and Flachowsky, G. 2004. Effects of Fusarium toxin contaminated maize in diets for piglets and the efficacy of a modified aluminosilicate as a detoxifying agent. Submitted.

- Drochner, W. Heckötter, E. and Scholz, H. 1984. Aktuelle Ergebnisse aus der tierärztlichen Fütterungsberatung. 4. Die Zusammensetzung von Grünfütter und Silagen für Wiederkäuer; Untersuchung von Einzelfuttermittel für Wiederkäuer in Problembeständen (ein Erfahrungsbericht). Deutsche Tierärztliche Wochenschrift 91, 61-66.
- EC (European Commission) 2000. Draft Commission Recommendation on the reduction of the presence of deoxynivalenol in cereals and cereal products. SANCO/1925/00-rev-1.
- EC (European Community) 2003. SCOOP Task 3.2.10. Collection of occurrence data on Fusarium toxins in foods and assessment of dietary intake by the population of EU Member States. April 2003. <http://europa.eu/comm/food/fs/scoop/task3210.pdf>
- Edwards, S.G., Pirgozliev, S.R., Hare, M.C., and Jenkinson, P., 2001. Quantification of trichothecene-producing Fusarium species in harvested grain by competitive PCR to determine efficacies of fungicides against fusarium head blight of winter wheat. Applied and Environmental Microbiology, 67, 1575-1580
- Ehrlich, K.C. and Daigle, K.W. 1987. Protein synthesis inhibition by 8-oxo-12,13-epoxytrichothecenes. Biochim. Biophys. Acta 923, 206-13.
- El-Banna, A.A., Hamilton, R.M., Scott, P.M. and Trenholm, H.L. 1983. Nontransmission of deoxynivalenol (vomitoxin) to eggs and meat in chickens fed deoxynivalenol-contaminated diets. J. Agric. Food Chem. 31, 1381-1384.
- Eriksen, G.S. and Alexander, J. 1998. Fusarium toxins in cereals- a risk assessment. Nordic Council of Ministers, TemaNord 502, Copenhagen, Denmark.
- Eriksen, G.S., Pettersson, H. and Lindberg, J.E. 2003. Absorption, metabolism and excretion of 3-acetyl DON in pigs. Arch. Tierernähr. 57, 335-345.
- Eriksen, G.S. and Pettersson, H. 2004. Toxicological evaluation of trichothecenes in animal feed. Animal Feed Sci. Tech. (in press)
- Farnworth, E.R., Hamilton, R.M., Thompson, B.K. and Trenholm, H.L. 1983. Liver lipid levels in White Leghorn hens fed diets that contained wheat contaminated by deoxynivalenol (vomitoxin). Poult. Sci. 62, 832-6.
- FAO (Food and Agriculture Organization) 1997. Worldwide Regulations for Mycotoxins 1995. A compendium. FAO Food and Nutrition Paper 64, Rome, Italy.
- FAO (Food and Agriculture Organization) 2004. Worldwide Regulations for Mycotoxins 2003. A compendium. FAO Food and Nutrition Paper, Rome, Italy. In press.
- FAPAS® (Food Analysis Performance Assessment Scheme) 2000. Fusarium Toxins, FAPAS® Series 22 Round 02, December 2000, Report 2202, Central Science Laboratory, Sand Hutton, York, UK.
- FAPAS® (Food Analysis Performance Assessment Scheme, 2002. Fusarium Toxins, FAPAS® Series 22 Round 07, June 2002, Report 2207, Central Science Laboratory, Sand Hutton, York, UK.
- FAPAS® (Food Analysis Performance Assessment Scheme) 2003a. Fusarium Toxins, FAPAS® Series 22 Round 10, April 2003-May 2003, Report 2210, Central Science Laboratory, Sand Hutton, York, UK.
- FAPAS® (Food Analysis Performance Assessment Scheme) 2003b. Fusarium Toxins, FAPAS® Series 22 Round 12, October to December 2003, Report 2212, Central Science Laboratory, Sand Hutton, York, UK.
- Fioramonti, J., Dupuy, C., Dupuy, J. and Bueno, L., 1993. The mycotoxin deoxynivalenol delays gastric emptying through serotonin-3 receptors in rodents. J. Pharmacol 266, 255-260.

- Fitzpatrick, D.W., Boyd, K.E., Wilson, L.M. and Wilson, J.R. 1988. Effect of the trichothecene deoxynivalenol on brain biogenic monoamines concentrations in rats and chickens. *J. Environ. Sci. Health. B* 23, 159-170.
- Forsyth, D.M., Yoshizawa, T., Morooka, N. and Tuite, J. 1977. Emetic and refusal activity of deoxynivalenol to swine. *Appl. Environ. Microbiol.* 34, 547-52.
- Foster, B.C., Trenholm, H.L., Friend, D.W., Thompson, B.K. and Hartin, K.E. 1986. Evaluation of different sources of deoxynivalenol (vomitoxin) fed to swine. *Can. J. Anim. Sci.* 66, 1149-1154.
- Friend, D.W., Trenholm, H.L., Elliot, J.I., Thompson, B.K. and Hartin, K.E. 1982. Effect of feeding vomitoxin-contaminated wheat to pigs. *Can. J. Anim. Sci.* 62, 1211-1222.
- Friend, D.W., Trenholm, H.L., Fiser, P.S., Thompson, B.K. and Hartin, K.E. 1983. Effect on dam performance and fetal development of deoxynivalenol (vomitoxin) contaminated wheat in the diet of pregnant gilts [Physiological or toxicological effect]. *Can. J. Anim. Sci.* 63, 689-698.
- Friend, D.W., Trenholm, H.L., Young, J.C., Thompson, B.K. and Hartin, K.E., 1984. Effect of adding potential vomitoxin (deoxynivalenol) detoxicants or a *F. graminearum* inoculated corn supplement to wheat diets fed to pigs. *Can. J. Anim. Sci.* 64, 733-741.
- Friend, D.W., Thompson, B.K., Trenholm, H.L., Hartin, K.E. and Prelusky, D.B. 1986a. Effects of feeding deoxynivalenol (DON)-contaminated wheat diets to pregnant and lactating gilts and on their progeny. *Can. J. Anim. Sci.* 66, 229-236.
- Friend, D.W., Trenholm, H.L., Thompson, B.K., Prelusky, D.B. and Hartin, K.E. 1986b. Effect of deoxynivalenol (DON)-contaminated diet fed to growing-finishing pigs on their performance at market weight, nitrogen retention and DON excretion. *Can. J. Anim. Sci.* 66, 1075-1085.
- Friend, D.W., Thompson, B.K., Trenholm, H.L., Boermans, H.J., Hartin, K.E. and Panich, P.L., 1992. Toxicity of T-2 toxin and its interaction with deoxynivalenol when fed to young pigs. *Can. J. Anim. Sci.*, 72, 703-711.
- Götz-Schröm, S., Schollenberger, M., Lauber, U., Muller, H.-M. and Drochner, W. 1998. Wirkung von reinem Deoxynivalenol bei Läuferschweinen - Ergebnisse. In: Wolff, J., Betsche, T. (Eds.). *Proceedings of the 20th German Workshop on Mycotoxins.* pp.171-175.
- Hamilton, R.M., Thompson, B.K., Trenholm, H.L., Fiser, P.S. and Greenhalgh, R. 1985. Effects of feeding white Leghorn hens diets that contain deoxynivalenol (vomitoxin)-contaminated wheat. *Poult. Sci.* 64, 1840-1852.
- Hart, L.P. and Braselton, W.E. 1983. Distribution of vomitoxin in dry milled fractions of wheat infected with *Gibberella zeae*. *J. Agric. Food Chem.* 31, 657-659.
- Harvey, R.B., Kubena, L.F., Corrier, D.E., Witzel, D.A., Phillips, T.D. and Heidelbaugh, N.D. 1986. Effects of deoxynivalenol in a wheat ration fed to growing lambs. *Am. J. Vet. Res.* 47, 1630-1632.
- Harvey, R.B., Kubena, L.F., Huff, W.E., Elissalde, M.H. and Phillips, T.D. 1991. Hematologic and immunologic toxicity of deoxynivalenol (DON)-contaminated diets to growing chickens. *Bull. Environ. Contam. Toxicol.* 46, 410-416.
- Harvey, R.B., Kubena, L.F., Rottinghaus, G.E., Turk, J.R., Casper, H.H. and Buckley, S.A., 1997. Moniliformin from *Fusarium fujikuroi* culture material and deoxynivalenol from naturally contaminated wheat incorporated into diets of broiler chicks. *Avian Dis.* 41, 957-963.
- Hochsteiner, W. and Schuh, M. 2000. Die Fusarientoxine Deoxynivalenol und Zearalenon in österreichischen Futtermitteln. *Mycotoxin Research* 16A, 58-61

- Hochsteiner, W. and Schuh, M. 2001. Zum Vorkommen der Fusarientoxine Desoxygenivalenol und Zearalenon in Österreichischen Futtermitteln im Zeitraum von 1995 bis 1999. Dtsch. Tierarztl. Wochenschr. 108, 19-23.
- Huff, W.E., Kubena, L.F., Harvey, R.B., Hagler, W.M., Swanson, S.P., Phillips, T.D. and Creger, C.R., 1986. Individual and combined effects of aflatoxin and deoxynivalenol (DON, vomitoxin) in broiler chickens. Poult. Sci. 65, 1291-1298.
- Hughes, D.M., Gahl, M.J., Graham, C.H. and Grieb, S.L. 1999. Overt signs of toxicity to dogs and cats of dietary deoxynivalenol. J. Anim. Sci. 77, 693-700.
- Hulan, H.W. and Proudfoot, F.G., 1982. Effects of feeding vomitoxin contaminated wheat on the performance of broiler chickens. Poult. Sci. 61, 1653-1659.
- Hörberg, H. 2001. Dispersal of mycotoxin producing *Fusarium* spp. Thesis for licentiate degree, Dept Ecology and Crop Production report series no 3., University of Agricultural Sciences, Uppsala.
- Ingalls, J.R. 1996. Influence of deoxynivalenol on feed consumption by dairy cows. Anim. Feed Sci. Technol. 60, 297-300.
- Islam, Z., Moon, Y.S., Zhou, H.R., King, L.E., Fraker, P.J., Pestka, J.J., 2002. Endotoxin potentiation of trichothecene-induced lymphocyte apoptosis is mediated by up-regulation of glucocorticoids. Toxicol. Appl. Pharmacol. 180, 43-55.
- IARC (International Agency for Research on Cancer) 1993. Monographs on the evaluation of carcinogenic risks to humans; Vol. 56: Some naturally occurring substances, food items and constituents, heterocyclic aromatic amines and mycotoxins. International Agency for Research on Cancer, World Health Organization, pp 397-444, Lyon, France.
- Johnson, P.J., Casteel, S.W. and Messer, N.T. 1997. Effect of feeding deoxynivalenol (vomitoxin)-contaminated barley to horses. J. Vet. Diagn. Invest. 9, 219-221.
- Jonker, M.A., van Egmond, H.P. and Stephany, R.W. 1999. Mycotoxins in food of animal origin: a review. CRL document 389002095. European Community Reference Laboratory on Residues, National Institute for Public Health & the Environment, Bilthoven, The Netherlands.
- Khera, K.S., Whalen, C., Angers, G. 1986. A teratology study on vomitoxin (4-deoxynivalenol) in rabbits. Food Chem. Toxicol. 24: 421-424.
- Krska, R., Baumgartner, S. and Josephs, R. 2001. The state-of-the-art in the analysis of type-A and -B trichothecene mycotoxins in cereals, foodstuffs and cultures. Fresenius J Anal Chem 371, 285-299.
- Kubena, L.F., Swanson, S.P., Harvey, R.B., Fletcher, O.J., Rowe, L.D. and Phillips, T.D. 1985. Effects of feeding deoxynivalenol (vomitoxin)-contaminated wheat to growing chicks. Poult. Sci. 64, 1649-1655.
- Kubena, L.F., Harvey, R.B., Corrier, D.E., Huff, W.E. and Phillips, T.D. 1987a. Effects of feeding deoxynivalenol (DON, Vomitoxin)-contaminated wheat to female white leghorn chickens from day old through egg production. Poult. Sci. 66, 1612-1618.
- Kubena, L.F., Harvey, R.B., Phillips, T.D., Holman, G.M. and Creger, C.R. 1987b. Effects of feeding mature White Leghorn hens diets that contain deoxynivalenol (vomitoxin). Poult. Sci. 66, 55-8.
- Kubena, L.F. and Harvey, R.B. 1988. Response of growing Leghorn chicks to deoxynivalenol-contaminated wheat. Poult. Sci. 67, 1778-80.
- Kubena, L.F., Huff, W.E., Harvey, R.B., Corrier, D.E., Phillips, T.D. and Creger, C.R. 1988. Influence of ochratoxin A and deoxynivalenol on growing broiler chicks. Poult. Sci. 67, 253-60.

- Kubena, L.F., Huff, W.E., Harvey, R.B., Phipps, D. and Rottinghaus, G.E. 1989. Individual and combined toxicity of deoxynivalenol and T-2 toxin in broiler chicks. *Poult. Sci.* 68, 622-626.
- Langseth, W. and Rundberget, T. 1998. Instrumental methods for determination of non-macrocytic trichothecenes in cereals, foodstuffs and cultures. *J Chrom A*, 918, 99-112.
- Lauren, D.R. and Ringrose, M.A. 1997. Determination of the fate of three *Fusarium* mycotoxins through wet-milling of maize using an improved HPLC analytical technique. *Food Addit. Contam.* 14, 435-443.
- Lee, U.S., Jang, H.S., Tanaka, T., Oh, Y.J., Cho, C.M. and Ueno, Y. 1987. Effect of milling on decontamination of *Fusarium* mycotoxins nivalenol, deoxynivalenol, and zearalenone in Korean wheat. *J. Agric. Food Chem.* 35, 126-129.
- Leitgeb, R., Lew, H., Wetscherek, W., Böhm, J. and Quinz, A. 1999. Einfluss von Fusarientoxinen auf die Mast- und Schlachtleistung von Broilern. *Die Bodenkultur*, 50, 57-66.
- Li, F.Q., Li, Y.W. and Yoshizawa, T. 2002. *Fusarium* toxins in wheat from an areas in Henan Province, PRE China, with a previous human mould intoxication episode. *Food Addit. Contam.*, 19. 163-166.
- Lun, A.K., Young, L.G. and Lumsden, J.H. 1985. The effects of vomitoxin and feed intake on the performance and blood characteristics of young pigs. *J. Anim. Sci.* 61, 1178-85.
- Lun, A.K., Young, L.G., Moran, E.T., Hunter, D.B. and Rodriguez, J.P. 1986. Effects of feeding hens a high level of vomitoxin-contaminated corn on performance and tissue residues. *Poult. Sci.* 65, 1095-1099.
- Lusky, K., Goebel, R., Tesch, D., Tenner, G., Haider, W., Krueger, M. and Lippert, A. 1998. Studies on the effects of ochratoxin A and deoxynivalenol toxicity on the health of pigs and tissue residue concentrations. *Tierarztl. Umsch.* 53, 623-630.
- MacDonald, S., Prickett, T.J., Wildey, K.B. and Chan, D. 2004. Survey of ochratoxin A and deoxynivalenol in stored grains from the 1999 harvest in the UK. *Food Addit Contam.* 2004 Feb;21(2):172-81.
- Marpegan, M.R., Perfumo, C.J., Godoy, H.M., Sala de Miguel, M., Diaz, E. and Risso, M.A., 1988. Feed refusal of pigs caused by *Fusarium* mycotoxins in Argentina. *Journal Of Veterinary Medicine Series A* 35, 610-616.
- Matthäus, K., Dänicke, S., Strumpf, A., Valenta, H., Zieseniß, H. and Flachowsky, G., 2004. Progression of the mycotoxin and nutrient concentration in wheat after inoculation with *Fusarium culmorum*. *Arch. Anim. Nutr.* 58, 19-35.
- Moon, Y. and Pestka, J.J. 2002. Vomitoxin-induced cyclooxygenase-2 gene expression in macrophage mediated by activation of ERK and p38 but not JNK mitogen-activated protein kinases. *Toxicol. Sci.* 69, 373-382.
- Moran, E.T., Hunter, B., Ferket, P., Young, L.G. and McGirr, L.G. 1982. High tolerance of broilers to vomitoxin from corn infected with *Fusarium graminearum*. *Poult. Sci.* 61, 1828-31.
- Müller, H.M. and Schwadorf, K. 1993. A Survey of the Natural Occurrence of *Fusarium* Toxins in Wheat Grown in a Southwestern Area of Germany. *Mycopathologia* 121, 115-121.
- Oldenburg, E. Lepschy, J. Valenta, H. und Weissbach, F. 1996. Fusarientoxine in Silomais – Abhängigkeit von sorte und standort. *Proceedings 18 Mykotoxin Workshop, Kulmbach*, 10-12 Juni 1996, 174-179.
- Oldenburg, E. and Höppner, F. 2003. *Fusarium* mycotoxins in forage maize – Occurrence, Risk assessment, Minimization. *Mycotoxin Research* 19, 43-46.

- Ouyang, Y.L., Azcona-Olivera, J.I., Murtha, J., Pestka, J.J. 1996. Vomitoxin-mediated IL-2, IL-4, and IL-5 superinduction in murine CD4+T cells stimulated with phorbol ester and calcium ionophore: relation to kinetics of proliferation. *Toxicol. Appl. Pharmacol.* 138, 324-334.
- Overnes, G., Matre, T., Sivertsen, T., Larsen, H.J.S., Langseth, W., Reitan, L.J. and Jansen, J.H. 1997. Effects of diets with graded levels of naturally deoxynivalenol-contaminated oats on immune response in growing pigs. *Journal of Veterinary Medicine Series A. Animal Physiology, Pathology, and Clinical Veterinary Medicine* 44, 539-550.
- Patey, A.L. and Gilbert, J. 1989. Fate of Fusarium mycotoxins in cereals during food processing and methods for their detoxification. In: Chelkowski, J (ed). *Fusarium Mycotoxins, Taxonomy and Pathogenicity*, Elsevier, Amsterdam, the Netherlands, pp 399-420.
- Pestka, J.J., Yan, D. and King, L.E. 1994. Flow cytometric analysis of the effects of in vitro exposure to vomitoxin (deoxynivalenol) of apoptosis in murine T, B and IgA+ cells. *Food Chem. Toxicol.* 32, 1125-1136.
- Pestka, J.J. and Zhou, H.R. 2000. Interleukin-6-deficient mice refractory to IgA dysregulation but not anorexia induction by vomitoxin (deoxynivalenol) ingestion. *Food Chem Toxicol.* 38,565-75.
- Pestka, J.J. and Zhou, H.R. 2003. Hck and PKR-dependent mitogen-activated protein kinase phosphorylation and AP-1, C/EBP and NF- κ B activation precede deoxynivalenol-induced TNF- α and MPI-2 expression. *Toxicologist* 72, 121
- Placinta, C.M., D'Mello, J.P.F., and Macdonald, A.M.C. 1999. A review of worldwide contamination of cereal grains and animal feed with Fusarium mycotoxins. *Animal Feed Science and Technology*, 78, 21-37.
- Poapolathep, A., Ohtsuka, R., Kiatipattanasakul, W., Ishigami, N., Nakayama, H. and Doi, K. 2002. Nivalenol-induced apoptosis in thymus, spleen and Peyer's patches of mice. *Exp. Toxicol. Pathol.* 53, 441-446.
- Poapolathep, A., Nagata, T., Suzuki, H., Kumagai, S. and Doi, K. 2003. Development of early apoptosis and changes in lymphocyte subsets in lymphoid organs of mice orally inoculated with nivalenol. *Exp. Mol. Pathol.* 75, 74-79.
- Pollmann, D.S., Koch, B.A., Seitz, L.M., Mohr, H.E. and Kennedy, G.A. 1985. Deoxynivalenol-contaminated wheat in swine diets. *J. Anim. Sci.* 60, 239-247.
- Prelusky, D.B., Trenholm, H.L., Lawrence, G.A. and Scott, P.M. 1984. Nontransmission of deoxynivalenol (vomitoxin) to milk following oral administration to dairy cows. *J. Environ. Sci. Health. B* 19, 593-609.
- Prelusky, D.B., Hamilton, R.M., Trenholm, H.L. and Miller, J.D. 1986a. Tissue distribution and excretion of radioactivity following administration of ¹⁴C-labeled deoxynivalenol to White Leghorn hens. *Fundam. Appl. Toxicol.* 7, 635-45.
- Prelusky, D.B., Veira, D.M., Trenholm, H.L. and Hartin, K.E. 1986b. Excretion profiles of the mycotoxin deoxynivalenol, following oral and intravenous administration to sheep. *Fundam. Appl. Toxicol.* 6, 356-363.
- Prelusky, D.B., Trenholm, H.L., Hamilton, R.M.G. and Miller, J.D. 1987a. Transmission of [¹⁴C]deoxynivalenol to eggs following oral administration to laying hens. *J. Agric. Food Chem.* 35, 182-186.
- Prelusky, D.B., Veira, D.M., Trenholm, H.L. and Foster, B.C. 1987b. Metabolic fate and elimination in milk, urine and bile of deoxynivalenol following administration to lactating sheep. *J. Environ. Sci. Health. B* 22, 125-148.
- Prelusky DB, Hartin KE, Trenholm HL, Miller JD. 1988. Pharmacokinetic fate of ¹⁴C-labeled deoxynivalenol in swine. *Fundam Appl Toxicol.* Feb;10(2):276-86.

- Prelusky, D.B., Hamilton, R.M. and Trenholm, H.L. 1989. Transmission of residues to eggs following long-term administration of ¹⁴C-labelled deoxynivalenol to laying hens. *Poult. Sci.* 68, 744-748.
- Prelusky, D.B. and Trenholm, H.L. 1991. Tissue distribution of deoxynivalenol in swine dosed intravenously. *J. Agric. Food Chem.* 39, 748-751.
- Prelusky, D.B. and Trenholm, H.L. 1992. Nonaccumulation of residues in swine tissue following extended consumption of deoxynivalenol-contaminated diets. *J. Food Sci.* 57, 801-802.
- Prelusky, D.B., Gerdes, R.G., Underhill, K.L., Rotter, B.A., Jui, P.Y. and Trenholm, H.L. 1994. Effects of low-level dietary deoxynivalenol on haematological and clinical parameters of the pig. *Natur. Toxins* 2, 97-104.
- Prelusky, D.B., 1997. Effect of intraperitoneal infusion of deoxynivalenol on feed consumption and weight gain in the pig. *Natur. Toxins* 5, 121-125.
- Rafai, P., Bata, A., Jakab, L., and Vanyi, A., 2000. Evaluation of mycotoxin-contaminated cereals for their use in animal feeds in Hungary. *Food Additives and Contaminants*, 17, 799-808.
- Richter, W.I.F. 1989. Investigations of vomitoxin (deoxynivalenol) contaminated diets fed to growing-finishing pigs. *Wirtschaftseigene Futter*, 35, 126-136.
- Rotter, R.G., Thompson, B.K., Trenholm, H.L., Prelusky, D.B., Hartin, K.E. and Miller, J.D. 1992. A preliminary examination of potential interactions between deoxynivalenol (don) and other selected fusarium metabolites in growing pigs. *Can. J. Anim. Sci.* 72, 107-116.
- Rotter, B.A., Thompson, B.K., Lessard, M., Trenholm, H.L. and Tryphonas, H. 1994. Influence of low-level exposure to Fusarium mycotoxins on selected immunological and hematological parameters in young swine. *Fundam. Appl. Toxicol.* 23, 117-124.
- Rotter, B.A., Thompson, B.K. and Lessard, M. 1995. Effects of deoxynivalenol-contaminated diet on performance and blood parameters in growing swine. *Can. J. Anim. Sci.* 75, 297-302.
- Rotter, B.A., Prelusky, D.B. and Pestka, J.J. 1996. Toxicology of deoxynivalenol (vomitoxin). *J. Toxicol. Environ. Health* 48, 1-34.
- SCF (Scientific Committee on Food) 1999. Opinion on fusarium toxins, Part 1: Deoxynivalenol (DON) expressed on 2 December 1999. European Commission SCF/CS/CNTM/MYC/19 Final, http://europa.eu/comm/food/fs/sc/scf/out44_en.pdf
- SCF (Scientific Committee on Food) 2002. Opinion of the Scientific Committee on Food on Fusarium toxins. Part 6: Group evaluation of T-2 toxin, HT-2 toxin, nivalenol and deoxynivalenol, adopted on 26 Februari 2002. European Commission SCF/CS/CNTM/MYC/27 Final, http://europa.eu/comm/food/fs/sc/scf/out123_en.pdf.
- Schuh, M., 1982. The importance of fusariotoxicoses in Austrian domestic animals. *Proc. Animal Production, Uppsala*, 390-394.
- Schwadorf, K. and Müller, H-M. 1995. Diseases in farm animals in relation to Fusarium toxins in feed (in German). *Landbauforschung Völknerode* 157, 100-103.
- Scott, P.M., Kanhere, S.R., Lau, P.-Y., Dexter, J.E. and Greenhalgh, R. 1983. Effects of Experimental Flour Milling and Breadbaking on Retention of Deoxynivalenol (Vomitoxin) in Hard Red Spring Wheat, *Cereal Chemistry* 60 (6); 421-424.
- Scott, P.M., Kanhere, S.R., Dexter, J.E., Brennan, P.W. and Trenholm, H.L. 1984. Distribution of the trichothecene mycotoxin deoxynivalenol (vomitoxin) during the milling of naturally contaminated hard red spring wheat and its fate in baked products, *Food Additives and Contaminants* 1 (4); 313-323.

- Scudamore, K.A., Nawaz, S., and Hetmanski, M.T.. 1998. Mycotoxins in ingredients of animal feedings: II. Determination of mycotoxins in maize and maize products. *Food Additives and Contaminants*, 15, 30-55.
- Seitz, L.M., Yamazaki, W.T., Clements, R.L., Mohr, H.E. and Andrews, L. 1985. Distribution of deoxynivalenol in soft wheat mill streams. *Cereal Chem.* 62, 467-469.
- Seitz, L.M., Eustace, W.D., Mohr, H.E., Shogren, M.D. and Yamazaki, W.T. 1986. Cleaning, milling, and baking tests with hard red winter wheat containing deoxynivalenol. *Cereal Chem.* 63, 146-150.
- Shifrin, V.I. and Anderson, P. 1999. Trichothecene mycotoxins trigger a ribotoxic stress response that activates c-Jun N-terminal kinase and p38 mitogen-activated protein kinase and induces apoptosis. *J. Biol. Chem.* 274, 13985-13992.
- Shinozuka, J., Li, G.M., Kiatipattanasakul, W., Uetsuka, K., Nakayama, H. and Doi, K. 1997a. T-2 toxin-induced apoptosis in lymphoid organs of mice. *Exp. Toxicol. Pathol.* 49, 387-392.
- Shinozuka, J., Li, G.M., Uetsuka, K., Nakayama, H. and Doi, K. 1997b. Process of the development of T-2 toxin-induced apoptosis in the lymphoid organs of mice. *Exp. Anim.* 46, 117-126.
- Sudakin, D.L. 2003. Trichothecenes in the environment: relevance to human health. *Toxicol. Lett.* 143, 97-107.
- Swamy, H. V., Smith, T. K., Macdonald, E. J., Boermans, H. J., and Squires, E. J. 2002. Effects of feeding a blend of grains naturally contaminated with *Fusarium* mycotoxins on swine performance, brain regional neurochemistry, and serum chemistry and the efficacy of a polymeric glucomannan mycotoxin adsorbent. *J Anim Sci*, 80, 3257-3267.
- Swamy, H. V., Smith, T. K., Macdonald, E. J., Karrow, N. A., Woodward, B., and Boermans, H. J. 2003. Effects of feeding a blend of grains naturally contaminated with *Fusarium* mycotoxins on growth and immunological measurements of starter pigs, and the efficacy of a polymeric glucomannan mycotoxin adsorbent. *J Anim Sci*, 81, 2792-2803.
- Swanson, S.P., Nicoletti, J., Rood, H.D., Buck, W.B., Cote, L.M. and Yoshizawa, T. 1987. Metabolism of three trichothecene mycotoxins, T-2 toxin, diacetoxyscirpenol and deoxynivalenol, by bovine rumen microorganisms. *J. Chromatogr.* 414, 335-342.
- Thompson, W.L. and Wannemacher, R.W. 1986. Structure-function relationships of 12,13-epoxytrichothecene mycotoxins in cell culture: comparison to whole animal lethality. *Toxicon* 24, 985-94.
- Trenholm, H.L., Cochrane, W.P., Cohen, H., Elliot, J.I., Farnworth, E.R., Friend, D.W., Hamilton, R.M., Standish, J.F. and Thompson, B.K. 1983. Survey of vomitoxin contamination of 1980 Ontario white winter wheat crop: results of survey and feeding trials. *J. Assoc. Off. Anal. Chem.* 66, 92-7.
- Trenholm, H.L., Thompson, B.K., Hartin, K.E., Greenhalgh, R. and McAllister, A.J. 1985. Ingestion of vomitoxin (deoxynivalenol)-contaminated wheat by nonlactating dairy cows. *J. Dairy Sci.* 68, 1000-5.
- Trenholm, H.L., Foster, B.C., Charmley, L.L., Thompson, B.K., Hartin, K.E., Coppock, R.W. and Albassam, M.A. 1994. Effects of feeding diets containing *Fusarium* (naturally) contaminated wheat or pure deoxynivalenol (DON) in growing pigs. *Can. J. Anim. Sci.* 74, 361-369.
- Ueno, Y., Nakajima, M., Sakai, K., Ishii, K., Sato, N. and Shimada, N. 1973. Comparative toxicology of trichothecene mycotoxins: Inhibition of protein synthesis in animal cells. *J. Biochem.* 74, 285-296.
- Warner, R.L., Brooks, K. and Pestka, J.J. 1994. In vitro effects of vomitoxin (deoxynivalenol) on T-cell interleukin production and IgA secretion. *Food Chem. Toxicol.* 32, 617-625.

- WHO (World Health Organization) 2001. Safety evaluation of certain mycotoxins in food. Prepared by the Fifty-sixth meeting of the Joint FAO/WHO Expert Committee on Food Additives. WHO Food Additives Series, No. 47; FAO Food and Nutrition Paper 74, 2001.
- Williams, K.C., Blaney, B.J. and Magee, M.H. 1988. Responses of pigs fed wheat naturally infected with *Fusarium graminearum* and containing the mycotoxins 4-deoxynivalenol and zearalenone. *Aust. J. Agric. Res.* 39, 1095-1105.
- Wong, S.S., Zhou, H.R., Marin-Martinez, M.L., Brooks, K., Pestka, J.J., 1998. Modulation of IL-1beta, IL-6 and TNF- α secretion and mRNA expression by the trichothecene vomitoxin in the RAW 264.7 murine macrophage cell line. *Food Chem. Toxicol.* 36, 409-419.
- Wong, S.S., Zhou, H.R., Pestka, J.J., 2002. Effects of vomitoxin (deoxynivalenol) on the binding of transcription factors AP-1, NF- κ B, and NF-IL6 in RAW 264.7 macrophage cells. *J. Toxicol. Environ. Health Part A* 65, 101-120.
- World Health Organization (2002), 'Evaluation of certain mycotoxins in food'. Fifty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives, WHO Technical Report Series 906, World Health Organisation, Geneva, Switzerland.
- Young, L.G., McGirr, L., Valli, V.E., Lumsden, J.H. and Lun, A. 1983. Vomitoxin in corn fed to young pigs. *J. Anim. Sci.* 57, 655-64.
- Young, J.C., Fulcher, R.G., Hayhoe, J.H., Scott, P.M. and Dexter, J.E. 1984. Effect of milling and baking on deoxynivalenol (vomitoxin) content of eastern canadian wheats. *J. Agric. Food Chem.* 32, 659-664.
- Zhou, H.R., Yan, D., Pestka, J.J., 1997. Differential cytokine mRNA expression in mice after oral exposure to the trichothecene vomitoxin (deoxynivalenol): dose response and time course. *Toxicol. Appl. Pharmacol.* 144, 294-305.
- Zhou, H.R., Yan, D., Pestka, J.J., 1998. Induction of cytokine gene expression in mice after repeated and subchronic oral exposure to vomitoxin (deoxynivalenol): differential toxin-induced hyporesponsiveness and recovery. *Toxicol. Appl. Pharmacol.* 151, 347-358.

SCIENTIFIC PANEL MEMBERS

Jan Alexander, Herman Autrup, Denis Bard,, Angelo Carere, Lucio Guido Costa; Jean-Pierre Cravedi, Alessandro Di Domenico, Roberto Fanelli, Johanna Fink-Gremmels, John Gilbert, Philippe Grandjean, Niklas Johansson, Agneta Oskarsson, Andrew Renwick, Jirí Ruprich, Josef Schlatter, Greet Schoeters, Dieter Schrenk, Rolaf van Leeuwen, Philippe Verger.

ACKNOWLEDGEMENT

The Scientific Panel on Contaminants in the Food Chain wishes to thank Sven Dänicke, Hans van Egmond, Johanna Fink-Gremmels, John Gilbert, Jürgen Gropp, John Christian Larsen, Josef Leibetseder, Monica Olsen, Hans Pettersson and Ron Walker for the contributions to the draft opinion.

ANNEX**Table 1. Collation of data for DON in unprocessed cereals taken from SCOOP Report (EC, 2003)**

Country	Sample type	Survey Year	Total no of samples	Numbers of samples containing DON in range µg/kg			
				< 300	300 - 500	500 - 1000	>1000
Austria	maize	1996-98	151	104	19	16	12
	wheat	1999-01	166	123	20	8	15
	oats	1999-01	232	229	2	1	0
Belgium	wheat	2001-02	47	42	4	1	0
France	wheat	2001-02	57	53	3	0	1
	maize	2001	259	110	34	57	58
Germany	wheat	1999	26	20	4	2	0
Netherlands	barley	1998-01	40	39	0	1	0
	oats	1998-01	38	38	0	0	0
	maize	2000-01	137	12	26	54	45
	rye	1998-01	37	37	0	0	0
	wheat	1998-01	2583	1787	352	294	150
Norway	wheat	1990-01	993	909	49	28	7
	barley	1990	20	20	0	0	0
	rye	1990-96	64	64	0	0	0
	oats	1993-01	242	222	3	15	2
Portugal	wheat	2002	3	2	0	1	0
Sweden	wheat	1996-01	126	104	4	0	18
	oats	1996-01	36	36	0	0	0
	rye	1996-99	47	46	1	0	0
UK	barley	1999-01	153	153	0	0	0
	mixed	2000-01	29	28	0	1	0

1 **Table 2: Collation of data for DON in European grains taken from JECFA (WHO, 2001)**

Sample type	Total no. Samples	Mean (µg/kg)	Maximum level (µg/kg)
Barley	627	860	26,000
Maize	1300	11	19,000
Oats	828	140	2,600
Rye	295	39	1,300
Triticale	10	92	200
Wheat	10,765	310	21,000

Table 3. Summary of effects of exposing pigs to DON in feed (n= number of animals in each group)*(Table modified from Eriksen and Pettersson 2004).*

Animals	n	Conc. (mg/kg feed)	Toxin source	Expos. time	Effects	LOEL (ppm)	NOEL (ppm)	Ref
York shire (6 weeks)	6	0- 9	Pure	7 days	Reduced feed intake and weight gain	4		[1]
York shire (6 weeks)	7	0-3 ad. lib and pair fed controls	Cont. corn	28 days	Reduced feed intake and body-weight gain (first 7 days) Reduced feed intake and body-weight gain, decreased thyroid weight (not compared to pair-fed) Linearly decreased skin temp and thyroid wt, reduced feed intake, b.w. (trend significant, but no differences between groups), corrugated stomach, Improved feed efficiency, Increased serum T4, albumin and decreased serum α -globulin compared to ad lib control, but not to pair-fed. reduced anti-body response to SRBC after 1 and 2 weeks, but not after 3. The response delayed.	0.75 1.5	0.75 Not given	[2]
Landrace X Piet. (no age or b.w.)	9	0.3 - 1.2	Pure	8 weeks	No effect on weight gain, tendency towards reduced IGF-1 and IgA,		> 1.2	[3]
York shire (7-8 weeks old)	8	0, 4.0 ad. lib and pair fed controls	Cont. corn	42 days	Reduced feed intake (20 %) and b.w. gain (13 %), corrugated stomach compared to controls, temporary decreased serum protein after 2 and 3 weeks, temporary reduced β -globulin weeks 2-4.	4.0		[4]
Landrace (ca 25 kg b.w.)	8	0.1 - 4.5	Cont. oat	8 weeks	Transient reduced feed consumption and weight gain Decreased weight gain whole period decreased feed utilisation	2.3 4.5	1.14 2.3	[5]
Landrace (ca 21 kg)	17-20	0.05 - 3.50	Cont. oat	Ca 3 months (21-100 kg b.w.)	Transient reduction in PCV, Decreased feed consumption Decreased serum calcium and phosphorus (< 5 %), Increased rel. liver weight	0.70 1.68	0.70 1.68	[6]
York shire (4-5 weeks)	4 4-5	0, 3.4 - 19.1 0 - 8.7	Pure or cont. wheat Cont. wheat	2 weeks 7 weeks	Reduced feed intake, Reduced b.w., (partially recovered if < 12 ppm), decreased total serum protein and phosphorus, reduced alkaline phosphatase, increased rel. stomach and adrenal weights, decreased rel kidney wt, Reduced feed intake and b.w.. Increased rel. liver weight Increased rel stomach wt	3.4 3.9	5.9 10.7 5.0	[7]

Animals	n	Conc. (mg/kg feed)	Toxin source	Exposure time	Effects	LOEL (ppm)	NOEL (ppm)	Ref
Landrace (12-13 weeks)	6	0, 2.5	Cont. corn	5 weeks	Reduced feed intake and weight gain, significant changes in stomach mucosa	2.5		[8]
York-shire (12-15 weeks)	5	0, 6	Pure toxin	21 days	Reduced feed intake, weight gain and slightly reduced feed efficiency. Lesions in stomach	6		[9]
York-shire (9-10 weeks)	3	0 - 5.2	Cont. maize and pure DON	7 weeks	Reduced feed intake and b.w., increased rel stomach and urinary bladder wts, stomach lesions		-	[10]
? (16-18 kg b.w.)	15-16	0 - 5.26	Cont. wheat	90 - 110 days (ca 17 - 90 kg b.w.)	Reduced feed intake and increased age at slaughter wt. No significant increase in wt of kidney, liver and uterus. No effect on feed efficiency. Stomach lesions		2.89	[11]
York-shire (ca 25 kg)	12	3.7, 4.2	Cont. wheat or maize	7 weeks	Decreased feed consumption and wt		-	[12]
? (7-9 kg)	3-4	0.14-875	Cont. corn	4-21 days (4 trials)	Vomiting Reduced feed intake and b.w. Increased serum protein, albumin, cholesterol, Decreased serum P, glucose, alkaline phosphatase.	20 1.34 9.0	9.0	[13]
? (cross-breed 5 week)	8-10	0.7 - 5.8	Cont. corn	4 week	Reduced feed intake and b.w. (male more than female. Reversed in females when given control feed). Reddening of mucosa in stomach and small intestines and oedema in the mesenteric lymph nodes.	3.1		[14]
? (8-9 kg)	10	10.5 ad. lib and pair fed controls	Cont. corn	21 days	Reduced feed consumption and b.w., Reduced feed efficiency (P<0.07) compared to ad lib control, but no difference to pair-fed control. Decreased serum hematocrit, haemoglobin, glucose, P compared to ad. lib control., but higher haemoglobin and no other significant change compared to pair-fed control		-	[15]
? (ca 9 kg)	8	0 - 2.8	Cont. Wheat	2 or 3 weeks	Reduced feed intake and b.w.. (No difference in wt gain when fed a clean diet after 4 weeks exposure).		0.9	[16]
? (ca 61 kg)	4	0 - 4.2	Cont. wheat	42 days	Reduced feed intake and b.w. gain. No effect on organ weight,		0.9	

Animals	n	Conc. (mg/kg feed)	Toxin source	Exposure time	Effects	LOEL (ppm)	NOEL (ppm)	Ref
? (45 kg)	4	0, 6.3 7.2	Cont. corn and pure Pure	4 days	Reduced feed intake Reduced feed intake	6.3 3.6		[17]
? (20 kg)	4	0 - 40	Pure	4 days	Dose-dependent reduction in feed intake	3.6		
? (20 kg)	3	0 - 16	Cont. corn	3 days	Reduced feed intake, reduced weight gain.			
? (20 kg)		3.6, 12.5		3 days	Reduced feed intake, reduced weight gain. (Pigs receiving 3.6 mg DON/kg naturally contam corn had a lower feed intake and average daily weight gain than all pigs receiving pure toxin)	3.6		
? (ca 24 kg)	6 or 12	< 0.05 - 11.0	Cont. Wheat	14 weeks	Reduced feed intake, vomiting Reduced weight gain:feed ratio		1.6 1.6	[18]
Landrace (ca 25 kg)	8	0.6 - 4.7	Cont. oat	About 100 days (25 - 100 kg)	Reduced feed intake, feed conversion and reduced antibody response toward tetanus toxoid (but not 4 other antigens, and only after 9 weeks, not after 3 and 6). Increased lymphocyte response toward PHA mitogen. Interstitial hepatitis in liver (p ca 0.05)		0.6 ? ?	[19]
Landrace (from 22 kg)	15	0.03, 6.0,	Cont. wheat	12 weeks	Reduced feed consumption and weight gain (not in the enhanced diet)	6.0		[20]
? (80-90 kg)	11	8	Cont. wheat	11 days	Reduced feed consumption compared to previous 5 days. Slowly increasing from day 6. Degeneration of hepatocytes, degenerative changes in renal tubular epithelium and eosinophilic infiltration in lymphatic organs.	8		[21]

Animals	n	Conc. (mg/kg feed)	Toxin source	Exposure time	Effects	LOEL (ppm)	NOEL (ppm)	Ref
Yorkshire (ca 39 kg)	6	0.05, 0.75	Cont. Wheat	21 days	Reduced feed intake and weight gain first 3 days. No difference later, but the loss was never recovered.		0.75	[22]
Yorkshire (ca 75 kg)	6	0.05-0.75	Cont. wheat	21 days	Dose-dependent reduction in feed intake and weight gain first 3 days, which were not recovered. Discoloration of oesophageal region of the stomach ($p > 0.05$).	0.38		
Yorkshire (ca 43 kg)	4	(0.05 - 0.75)	Cont. wheat	7 weeks	Reduced feed intake and reduced feed:gain ratio.	(only trend)		
Yorkshire (ca 21 kg)	4	(0.05 - 0.75)		4 weeks	Reduced wt gain, significant trend for wt gain and feed intake.	(only trend)		
Yorkshire (age and size not specified)	6	0 - 0.7	Cont. wheat	21 days	Reduced feed intake and average wt gain especially days 1-3.	0.35		[23]
Yorkshire (ca 30 kg)	10	0.20, 5.08	Cont. wheat	5 weeks	Reduced feed intake and wt gain.	5.08		[24]
Yorkshire (9 weeks)	6	0 - 3.0	Cont. corn and pure toxin	32 days	Reduced feed intake and wt gain (whole period for nat contam, only 2 days for pure toxin). Significant decrease in serum gammaglobulin content and trend towards a decrease in total globulin content. No significant pathological changes in stomach region, but trend towards more mucosal folding and thickening of oesophageal region tissue with increasing DON.		1.0	[25]
? (60 kg)	6 (2 control)	0, 1.0	Pure	90 days	No effect on feed intake or b.w. or b.w. gain or other parameters measured.		> 1.0	[26]
? (age and size not specified)	?	0 - 5	?		Reduced feed intake Changed feed: wt gain ratio	1	1	[27]

Animals	n	Conc. (mg/kg feed)	Toxin source	Exposure time	Effects	LOEL (ppm)	NOEL (ppm)	Ref
? (70-83 days old). ?	6	0 - 1.4	Cont. wheat	100-125 days	No effect on feed intake, wt gain, or other parameters measured.		> 1.4	
(ca 30 kg)	12 - 18	0.2 - 2.8	Cont. wheat	(30 to 100 kg b.w.)	No effect on feed consumption, wt gain or other parameters measured		> 2.8	
Yorkshire (ca 90 kg)	12	0.1 - 3.5	Cont. wheat	50-54 days (from 178 days, during pregnancy)	Reduced maternal b.w. and feed intake, Significant trend towards reduced foetal weights and foetal lengths		1.7 1.7	[29]
Four-strain male and female hybrides	12	2.4-3.1	Cont. wheat	98 days (~30 - 110 kg body weight)	Reduced feed intake and body weight gain, improved digestibility of nutrients, no effects on clinical chemistry, detection of DON in serum, urine was the major route of DON-excretion, traces of de-epoxy-DON in urine.			[30]
Four-strain male and female hybrides	16	Period 1: 2.6, 4.1	Cont. wheat	14 days (~28 - 36 kg body weight)	Pigs fed the highest DON-concentration reduced feed consumption to approximately 50 % of the control group within 2 days after the beginning of the experiment, some individual pigs completely refused feed.			[31]
Four-strain male and female hybrides	18	0.2, 0.7, 1.2, 2.5, 3.7	Cont. wheat	70 days (~56 - 103 kg body weight)	No significant effects on performance with a tendency of a dose-independent decrease in performance due to DON-presence in the diets, no effects on clinical chemistry, linearly related increase of DON-concentration in serum, no effects on nutrient digestibility.			[32]
Four-strain female hybrides	20	0.2 - 3.9	Cont. corn	35 days (12.5 - 32.5 kg)	Feed intake and body weight gain significantly decreased and total serum protein significantly decreased at 3.9 mg/kg Serum activity of GLDH significantly decreased at 0.8 mg/kg or more No or no consistent effects on organ weights of the digestive tract or related organs and immune globulin concentration in the serum Linearly related increase of DON-concentration in the serum			[33]

Animals	n	Conc. (mg/kg feed)	Toxin source	Exposure time	Effects	LOEL (ppm)	NOEL (ppm)	Ref
Four-strain female hybrides	20	2.3	Cont. corn	35 days (10.5 - 27.5 kg)	Feed intake, body weight gain and feed to gain ratio significantly decreased Relative weight of stomach and heart significantly increased Serum albumin concentration and activity of GLDH significantly decreased			[34]
	12	3.2		(9.7 - 21.4 kg)	Feed intake and body weight gain significantly decreased			
Yorkshire (10 kg)	35	4.6	Cont. corn and wheat	21 days	Feed intake and body weight gain significantly decreased Relative liver and kidney weights significantly decreased Significantly decreased concentrations of neurotransmitters in pons and hypothalamus Significantly increased serum concentrations of immune globulins A and M			[35]
Yorkshire (9.3 kg)	30	3.9 and 5.8 (with pair fed control)	Cont. corn and wheat	21 days	Feed intake and body weight gain decreased linearly Albumin : globulin ratio increased linearly, total serum protein and globulin concentrations significantly decreased (as compared to pair fed pigs) No response of organ weights, serum immune globulines, percentages of peripheral blood lymphocyte subsets and primary response to sheep red blood cells			[36]

References: 1 Prelusky (1997), 2 Rotter *et al.*, (1994), 3 Götz-Schröm *et al.*, (1998), 4 Rotter *et al.*, (1995), 5 Bergsjö *et al.*, (1992), 6 Bergsjö *et al.*, (1993b), 7 Trenholm *et al.*, (1994), 8 Friend *et al.*, (1992), 9 Rotter *et al.*, (1992), 10 Foster *et al.*, (1986), 11 Friend *et al.*, (1986a), 12 Friend *et al.*, (1986b), 13 Young *et al.*, (1983), 14 Cote *et al.*, (1985), 15 Lun *et al.*, (1985), 16 Pollmann *et al.*, (1985), 17 Forsyth *et al.*, (1977), 18 Williams *et al.*, (1988), 19 Overnes *et al.*, (1997), 20 Chavez and Rheaume, (1986), 21 Marpegan *et al.*, (1988), 22 Friend *et al.*, (1982), 23 Trenholm *et al.*, (1983), 24 Friend *et al.*, (1984), 25 Prelusky *et al.*, (1994), 26 Lusky *et al.*, (1998), 27 Schuh, (1982), 28 Richter, (1989), 29 Friend *et al.*, (1983), 30 Dänicke *et al.*, 2004e, 31 Dänicke *et al.*, 2004g, 32 Dänicke *et al.*, 2004f, 33 Döll *et al.*, (2003), 34 Döll *et al.*, (2004), 35 Swamy *et al.*, (2002), 36 Swamy *et al.*, (2003).

Table 4. Summary of effects of exposing chickens to DON in feed (n= number of animals in each group). Table modified from Eriksen and Pettersson 2004.

Animals	n	Conc. (mg/kg feed)	Toxin source	Exposure time	Effects	LOEL (ppm)	NOEL (ppm)	Ref.
Ross (day 1)	45	1.8-5.4	Cont. maize	37 days	Increased heart weight		3.6	[1]
Broiler chicks (day 1)	36	16	Cont. wheat	21 days	No effect on feed intake, weight gain or other parameters measured.		> 16	[2]
White Leghorn (day 1)	100	0, 18	Cont. wheat	12 weeks	Reduced b.w. after 4 and 8 weeks, but not 12 weeks, increased rel. gizzard weight, decreased haemoglobin at 4 weeks, but not after 8 and 12 weeks.	18		[3]
White Plymouth Rock x White Cornish (day 1)	240	0.1-3.40	Cont. oat	35 days	No effect on feed intake, weight gain or meat quality.		> 3.40	[4]
Hubbard x Hubbard (day 1)	0, 16	0, 16	Cont. wheat	3 weeks	Decreased b.w., increased feed:wt gain ratio, rel gizzard wt, and rel. bursa wt.	16		[5]
Hubbard x Hubbard (day 1)	60	0, 16 ad lib	Cont. wheat	3 weeks	Decreased b.w., increased feed: weight gain ration, increased rel. Gizzard weight, increased red blood cell count and serum phosphorus, decreased MCH, and glucose	16		[6]
White Leghorn (day 1)	51	0, 9, 18	Cont. wheat	35 days	Reduced liver weight, Increased gizzard weight, temporary decreased plasma triglycerides, glucose, increased creatinine, Decreased plasma Hgb and temporary RBC	9		[7]
White Leghorn (day 1 to egg production)	30	0, 18 ad lib	Cont. wheat	48 weeks	Small significant increase in shell wt, shell thickness, decrease in serum uric acid, glucose, triglycerides and cholesterol and increase in serum gammaglutamyltransferase and alkaline phosphatase	18		[8]

Animals	n	Conc. (mg/kg feed)	Toxin source	Exposure time	Effects	LOEL (ppm)	NOEL (ppm)	Ref.
White Leghorn (day 7)	50	0- 0.7	Cont. wheat	14 days	No significant effect on feed intake, wt gain, or other parameters measured (Control feed composition differed from contaminated).		> 0.7	[9]
White Leghorn (day 7)	50	3.1- 4.1	Cont. wheat	28 days	Increased feed intake, wt gain and feed:gain ratio. or other parameters measured (Control feed composition differed from contaminated).			
Broiler chicks (day 7)	50	0-0.7	Cont. wheat	14 days	No effect on feed intake, wt gain, r or other parameters measured (Control feed composition differed from contaminated).			
Broiler chicks (day 7)	50	0.1-4.1	Cont. wheat	28 days	Increased feed intake, wt gain, decreased feed:gain ratio. No apparent lesions in oral cavity, or effect on other parameters measured (Control feed composition differed from contaminated).			
White Leghorn	60,	0, 18	Cont. wheat	18 and 9 weeks	Reduced immune response to vaccine reduced mitogen-induced lymphoblastogenesis.	18		[10]
Hubbard X Hubbard and white Leghorn	10	0, 50	Pure toxin	9 weeks	Reduced response to mitogens in female broiler chickens. No effect in male broiler chickens or in leghorn chickens of either gender	50		
White mountain X Hubbard (6 days)	24	0- 210.0	Cont. maize	5 days	Increased feed conversion, Reversible dose-dependent increase in oral and gizzard epithelial lesions. Reduced feed intake.	49.4	116.1	[11]
Shaver (from hatching)	18 x 2	< 0.2 – 1.87	Cont. wheat	28 days	No effect on feed intake, weight gain, growth, liver or kidney.		> 1.89	[12]
White Leghorn (23 –26 weeks)	12	0.12-4.9	Cont. oat	70 days	Small increase in incidence of anomalies in chick offspring (in the two middle concentrations, but not the highest)	2.5		[13]

Animals	n	Conc. (mg/kg feed)	Toxin source	Exposure time	Effects	LOEL (ppm)	NOEL (ppm)	Ref.
White Leghorn (335 day old)	24	0, 18	Cont. wheat	112 days	Small significant decreased egg shell wt and shell % of total egg and increased albumin height	18		[14]
White Leghorn	24	20	Pure	6 days	Radioactive labeled DON found in the eggs with a maximum concentration the last day of exposure of 0.07 mg/kg with a rapid decline when switched to clean feed			[15]
Hubbard X Hubbard (day 1)	60	0, 16	Cont. wheat	3 weeks	Reduced growth, increased feed efficiency, increased relative gizzard weight, anaemia, decreased LDH, and serum triglycerides	16		[16]
White Leghorn, X Single Comb (26 weeks)	10	0, 83	Cont. wheat	27 days	Small erosions in the gizzard, No other pathological changes.	83		[17]
White Leghorn (192 days)	102	< 0.5 - 0.7	Cont. wheat	70 days	Decreased egg weight, shell weight, shell thickness (pair-wise comp not given).		?	[18]
White Leghorn	28, 56	0.2 - 4.9	Cont. wheat	24 weeks	No significant effects.		> 4.9	
Different types	3	0 - 0.70	Cont. wheat	86 or 135 days	Increased liver triglycerides and total liver lipid in 0.35, not 0.70			[19]

References: 1 Leitgeb *et al.*, (1999), 2 Harvey *et al.*, (1997), 3 Kubena and Harvey, (1988), 4 Bergsjö and Kaldhusdal., (1994), 5 Kubena *et al.*, (1989), 6 Kubena *et al.*, (1988), 7 Kubena *et al.*, (1985), 8 Kubena *et al.*, (1987a), 9 Hamilton *et al.*, (1985), 10 Harvey *et al.*, (1991), 11 Moran *et al.*, (1982), 12 Hulan and Proudfoot, (1982), 13 Bergsjö *et al.*, (1993a), 14 Kubena *et al.*, (1987b), 15 Prelusky *et al.*, (1987a), 16 Huff *et al.*, (1986), 17 Lun *et al.*, (1986), 18 Hamilton *et al.*, (1985), 19 Farnworth *et al.*, (1983).

Table 5. Carry-over of deoxynivalenol into animal tissues and foodstuffs of animal origin.

Species/ category	DON-dosage (mg/kg diet) ¹⁾	Duration (days)	DON and metabolites in tissues or foodstuffs (µg/kg)	Remarks	Reference
Broiler Laying hen	4-5	28-190	Eggs, leg and breast meat, liver, gizzard: DON < d.l.	d.l. 10 µg/kg; metabolites and conjugates n.d.	El Banna <i>et al.</i> , 1983
Broiler	0; 9; 18	1-35	Liver, kidneys, heart, breast and leg meat: DON < d.l.	d.l. 10 µg/kg; metabolites and conjugates n.d.	Kubena <i>et al.</i> , 1985
Laying hen	83	182	Eggs, liver, kidneys, muscle: DON < d.l. gizzard: DON 20	Metabolites and conjugates n.d.	Lun <i>et al.</i> , 1986
Laying hen	18	Six 28-day eggproduction periods	Eggs: DON < d.l.	d.l. 10 µg/kg	Kubena <i>et al.</i> 1987a
Intact and colostomised laying hens	³ H-DON: 0.1 mg/kg b.w.	Single bolus	Low radioactivity in blood, tissues and eggs		Lun <i>et al.</i> , 1989
Laying hen	¹⁴ C-DON: 2.2 mg/hen/day	Single bolus or 12	Low radioactivity in tissues; < 40 µg DON-equivalents/kg except in liver, kidney and spleen; No accumulation in edible tissues after 12 days feeding of the DON-spiked diet		Prelusky <i>et al.</i> , 1986a
Laying hen	¹⁴ C-DON; 2.2 mg/hen/day (= 20 mg/kg diet)	Single bolus or 12	After single bolus: in eggs max. 1.9 µg DON- equivalents/egg (0.09 % of the dose) After 12 day oral exposure: in eggs max. 4.2 µg DON-equivalents/egg, i.e. appr. 70 µg/kg	Only 10 % of the radioactivity identified as DON	Prelusky <i>et al.</i> , 1987a
Laying hen	¹⁴ C-DON: 5.5	65	Eggs: max. 1.7 µg DON-equivalents/egg		Prelusky <i>et al.</i> , 1989
Piglet (5 weeks old)	5.8	28	Liver, kidneys, muscle: DON-traces (< 50)	Metabolites and conjugates n.d.	Cote <i>et al.</i> , 1985
Piglet (8 kg b.w.)	1.2; 2.4; 3.6	21	Kidney: DON 19-23; Liver: DON 5-12	Slaughtering immediately after the last feeding; metabolites and conjugates n.d.	Pollman <i>et al.</i> , 1985
Pig (61 kg b.w.)	2.2	42	Tissues: DON < d.l.	Slaughtering 12-15 h after the last feeding; metabolites and conjugates n.d.	
Pig (25 kg b.w.)	6.0-7.6	21-49	Liver, kidney, adipose tissue: DON-traces (3, 5 and 7)	d.l.: 2 µg/kg fresh tissue; metabolites and conjugates n.d.	Prelusky and Trenholm, 1992
Pig (60 kg b.w.)	1.0	90	Blood and organs: DON < d.l.	Incubated with glucuronidase metabolites n.d.	Lusky <i>et al.</i> , 1998

Species/ category	DON-dosage (mg/kg diet) ¹	Duration (days)	DON and metabolites in tissues or foodstuffs (µg/kg)	Remarks	Reference
Lactating cow	1.7 mg/kg KG	Single bolus	Milk: DON-traces (<4) (without and with incubation with glucuronidase)	Metabolites n.d.	Prelusky <i>et al.</i> , 1984
Lactating cow	66	5	Milk: DON < d.l.; DOM-1: 2-26	d.l. in milk: 1 µg/L; conjugates in milk n.d.	Cote <i>et al.</i> , 1986
Lactating cow	0, 6, 12 mg/kg concentrate	70	Milk: DON and DOM-1 < 1	conjugates n.d.	Charmley <i>et al.</i> , 1993
Lactating ewe	a) 880 b) 330	3	a) Milk: DON max. 17, DOM-1 max. 205 b) Milk: DON max. 10, DOM-1 max. 125	DON and DOM-1 mainly as glucuronide conjugates	Prelusky <i>et al.</i> , 1987b

b.w. - body weight; n.d. - not determined; d.l. - detection limit; DON - deoxynivalenol; DOM-1 - de-Epoxy-DON

1) Air dry basis, if not otherwise stated.