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SCIENTIFIC COMMITTEE ON FOOD

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OPINION ON FUSARIUM TOXINS

Part 1: Deoxynivalenol (DON)

(expressed on 2 December 1999)

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Terms of reference

Although it is acknowledged that there are gaps in the toxicological information available, the Scientific Committee on Food is requested

- to assess the health risk associated with exposure to the different Fusarium toxins in cereals, taking into the account the current state of knowledge.
- to indicate, on the basis of current knowledge, which of these Fusarium toxins are of most concern for public health and for which there is an urgent need for further research and/or need for measures to reduce the presence of these toxins in cereals.
- to indicate, if possible, the nature of the toxicological studies to recommend in order to elucidate (more) completely the toxicology of these toxins.

In considering these issues the Committee is asked to take note, inter alia, of the comprehensive report "Fusarium toxins in cereals – a risk assessment" which has been prepared for the Nordic Council of Ministers.

Background

A variety of Fusarium fungi, which are common soil fungi, produce a number of different mycotoxins of the class of trichothecenes (T-2 toxin, HT-2 toxin, deoxynivalenol (DON) and nivalenol and some other toxins (zearalenone and fumonisins). The Fusarium fungi are probably the most prevalent toxin-producing fungi of the northern temperate regions and are commonly found on cereals grown in the temperate regions of America, Europe and Asia.

Fusarium toxins have been shown to cause a variety of toxic effects in both experimental animals and livestock. On some occasions toxins produced by Fusarium species have also been suspected to cause toxicity in humans.

Introduction

In the evaluation of Fusarium toxins the criteria for toxin selection have been:

- the toxins most commonly found in analytical surveys of cereals
- the toxins for which there is a minimum of toxicological data.

The first group of toxins to be evaluated is: deoxynivalenol, T-2 toxin, HT-2 toxin, nivalenol, fumonisin B1 and zearalenone.

The present evaluation deals with deoxynivalenol (DON); it is primarily based on the report prepared for the Nordic Council of Ministers "Fusarium toxins in cereals – a risk assessment" (Eriksen and Alexander, 1998) and the recent Dutch evaluation of deoxynivalenol (Baars et al., 1999).

Deoxynivalenol (DON)

Description

Deoxynivalenol is a mycotoxin produced by fungi of the *Fusarium* genus, *i.e. Fusarium culmorum* and *Fusarium graminearum*, which are abundant in various cereal crops (wheat, maize, barley, oats, and rye) and processed grains (malt, beer and bread). Chemically it belongs to trichothecenes. In contaminated cereals 3- and 15-acetyl DON can in significant amounts (10 – 20%) occur concomitantly with DON. The fungi producing trichothecenes are soil fungi and are important plant pathogens which grow on the crop in the field (cited in Eriksen and Alexander, 1998).

Chemistry

4-Deoxynivalenol (DON; vomitoxin, dehydronivalenol, RD-toxin): 12,13-epoxy-3, 4,15-trihydroxytrichotec-9-en-8-one, $C_{15}H_{20}O_6$, MW: 296,32, CAS no.: 51481-10-8.

The substance is a very stable compound, both during storage/milling and the processing/-cooking of food, and it does not degrade at high temperatures (cited in Rotter et al., 1996; Ehling et al., 1997; Eriksen and Alexander, 1998).

Biochemical mode of action

DON inhibits the synthesis of DNA and RNA and protein synthesis at the ribosomal level. The toxin has a haemolytic effect on erythrocytes. An acute dose of DON can induce vomiting (emesis) in pigs, whereas at lower concentrations in the diet it reduces growth and feed consumption (anorexia). Both effects, which are also seen with other trichothecene toxins, are thought to be mediated by affecting the serotonergic activity in the CNS or via peripheral actions on serotonin receptors (Rotter et al., 1996; Eriksen and Alexander, 1998).

Toxicokinetics

Ninety-six hours after administration of a single dose of radioactive-labelled DON (10 mg/kg body weight (b.w.)) to rats, 64% of the radioactivity was recovered in the faeces and 25% in the urine. After oral administration the major metabolic route is de-epoxidation to the corresponding methylene derivative. This metabolite was found in faeces, urine, plasma and

milk of lactating cows; in addition its presence was demonstrated after one-week incubation with gut microflora from cows and pigs, probably due to microbiological adaptation of the gut flora. In sheep and cows glucuronidation of DON was observed. Following a single dose of radiolabelled DON in pigs the half-life of DON was estimated to 4 hrs. and only trace levels were present at 24h.There is no toxicokinetic information from humans and no information from mice from which species data on long term toxicity exist (cited in Eriksen and Alexander, 1998).

Toxicity

Acute and subacute toxicity

Oral LD50 values of 78 and 46 mg/kg b.w. have been reported for B6C3F1 and DDY mice, respectively. Acute/subacute toxicity of DON is characterised by vomiting (vomiting is seen in pigs, whereas delayed gastric emptying has been observed in rats and mice), feed refusal, weight loss and diarrhoea. After acute intoxication necrosis in various tissues such as gastrointestinal tract, bone marrow and lymphoid tissues is also observed. The minimum emetic dose in pigs was 0.05 - 0.2 mg/kg b.w., when given orally. Pigs are sensitive to DON in their feed and a reduced feed uptake was seen at 1 – 2 mg DON/kg feed.

Oral doses of 0.05 – 1 mg/kg b.w. to mice and rats delayed gastric emptying in a dose-related manner and in mice gastric repulsion was partly inhibited at 1 mg/kg b.w. (Fioramenti et al., 1993 as cited in Eriksen and Alexander, 1998; Rotter et al., 1996 as cited in IARC, 1993; Eriksen and Alexander, 1998).

Subchronic toxicity

After subchronic oral exposure of various species (mouse, rat and pigs) several effects were found i.e. reduced feed intake, reduced weight gain, and changed levels in some blood parameters including serum immunoglobulins (see Eriksen and Alexander. Baars et al., 1999).

The lowest NOAELs were found in two studies in young pigs fed naturally contaminated feed containing up to 4 mg DON/kg for about 3 months, namely 1 and 1.7 mg/kg feed (equivalent to 0.04 and 0.06 mg/kg b.w., respectively). At higher doses of 2 – 4 mg/kg feed (equivalent to 0.08 – 0.16 mg/kg b.w.) reduced weight gain, increased liver weights and decreased concentrations of serum protein and albumin and temporary decreases in packed cell volume, serum calcium and phosphorus were observed. No statistically significant effect was found in other parameters measured (carcass quality, IgA, other blood parameters, or pathological changes in kidney, spleen, ileal Peyers patches, mesenteric lymph nodes and pancreas) (Bergsjø et al., 1992; 1993).

It was noted that the feed used in the studies with pigs was naturally contaminated, making it impossible to exclude the presence of other toxins, which may have contributed to the observed effects.

Chronic toxicity and carcinogenicity

In a chronic diet study with B6C3F1 mice the animals were administered 0, 1, 5 or 10 mg DON per kg feed, daily during 2 years (males: 0, 0.1, 0.5 or 1.1, females: 0, 0.1, 0.7 or 1.5 mg/kg b.w./day). Survival was not significantly changed. A significantly reduced weight gain was seen in males and females at 5 and 10 mg/kg feed. DON did not cause biologically relevant effects in haematological and clinical-chemical parameters. In females serum immunoglobulins showed some increase of IgA and IgG (<10%) at 5 and 10 mg/kg feed. Relative liver weights in males were increased at 5 and 10 mg/kg feed; at 10 mg/kg feed also the relative testes weights were significantly increased. At this latter dosage males showed decreased relative spleen weights. No increase in incidence of preneoplastic or neoplastic changes was observed. The NOAEL in this study is 1 mg/kg feed, corresponding to 0.1 mg/kg b.w./day (Iverson et al., 1995).

In a two-stage experiment with Sencar mice, DON did not show any characteristics of skin tumour initiation or promotion (Lambert et al., 1995).

The IARC classified DON in 1993 in Category 3, i.e., not classifiable as to its carcinogenicity to humans; however, at that time the negative chronic study in mice (Iversen et al., 1995) was not available.

Genotoxicity

DON did not show mutagenic activity in Ames tests with *Salmonella typhimurium*, both with and without S-9 activation systems, and in an in vitro UDS test using rat primary hepatocytes. Neither did DON induce gene mutations at the HPRT locus of V79 cells. DON enhanced cell transformation in mouse embryo cells *in vitro*, and induced clastogenic effects and inhibited gap-junctional intercellular communication in Chinese hamster V79 cells. It should be noted that DON inhibited the protein synthesis in Chinese hamster ovary cells *in vitro* in the same dose range as that inducing clastogenic effects (Hsia et al., 1988; Leatherman and Middlebrook, 1993; cited in Eriksen and Alexander, 1998; see also IARC, 1993).

There are no *in vivo* data.

Immunotoxicity

Studies with experimental animals demonstrated effects on the immune system, notably effects on IgA. There are indications for a suppression of humoral and cellular immunity, resulting in an increased susceptibility for infectious diseases, as shown in experimental studies with mice (cited in Eriksen and Alexander, 1998; Deijns et al., 1994). Regarding this increased susceptibility for infectious diseases a NOAEL of 0.25 mg/kg bw/day (Tryphonas et al., 1986) and a lowest-effect level of 0.22 mg/kg bw/day have been reported in studies with male Swiss-Webster and male Balb/C mice, respectively (cited in Eriksen and Alexander, 1998; Deijns et al., 1994; Baars et al., 1999). Perinatal investigations with respect to the immune system are not available.

Reproductive toxicity and teratogenicity

Oral studies in mice, rats and rabbits did not show teratogenic effects. Embryotoxic effects were observed in mice and rabbits at maternally toxic doses, i.e. ≥ 1 mg/kg b.w. Increase in

postnatal mortality was observed in mice with a NOAEL= 0.375 mg/kg b.w. (Khera et al.,1984). Absence and fusions of ribs were also noted in mice at the maternally toxic doses; the NOAEL was 0.5 mg/kg b.w. (Khera et al., 1982). In one study with rats a slight decrease in fertility was observed at 2 mg/kg b.w., the only dose tested, whereas in another rat study doses up to 1 mg/kg b.w. did not show any effect (Khera et al., 1982,1984, 1986; Eriksen and Alexander,1998, Baars et al., 1999).

Two studies exposing swine to DON in the diet during gestation are reported. Gilts fed diets containing 0.1-4.8 mg DON/kg feed did not exhibit overt maternal toxicity or decreased feed consumption, but 1-2 mg/kg feed (0.03-0.07 mg DON/kg b.w./day) caused reduced weight gain. No effect in number of offspring or survival or any deformities was observed. No effect of DON on reproduction was observed in doses lower than those leading to reduced weight gain (1-2 mg/kg feed) (Bergsjø et al., 1992, 1993; Eriksen and Alexander, 1998, Baars et al. 1999).

Effects in humans

In an epidemiological study, reporting human food poisoning caused by infected wheat in India in 1989 which affected an estimated 50,000 people, a NOAEL of 0.44 µg/kg b.w. was estimated, using an average intake of 67 g wheat products and a mean b.w. of 52 kg. The symptoms described include abdominal pain or a feeling of fullness in the abdomen, dizziness, headache, throat irritation, nausea, vomiting, diarrhoea, and blood in the stool. However, samples were collected four months after the outbreak, and the exposure was not limited to DON but included also other toxins, which leads to gross uncertainties in the estimated NOAEL (see Eriksen and Alexander, 1998).

Other cases of food poisoning related to Fusarium contaminated cereals have been reported. However, information on the amount of DON and other mycotoxins present as well as on consumption figures was scarce, so no exposure estimate can be made.

Evaluation and Conclusion

The general toxicity and the immunotoxicity of DON are considered to be the critical effects. Only one long-term feeding study is available. No increase in tumour frequency or other sign of carcinogenic effect were found in this study (Iverson et al., 1995).

There are no indications for carcinogenic and/or mutagenic properties of DON. Thus, the evaluation can be based on a NOAEL from the toxicity studies, applying an uncertainty factor.

The NOAELs reported for DON vary between 0.04-0.375 mg/kg b.w./day and are summarised in the Table. The quality of the studies and the relevance of the toxicological endpoints in the studies have been taken into account. The following NOAELs are to be considered:

Study	**Critical Effect**	**NOAEL (mg/kg bw/day)**	**Reference**
Mouse, chronic (2 years)	Reduced growth	0.1	Iverson et al., 1995

Mouse, immunotoxicity	Increased susceptibility to infections	0.25	Tryphonas et al., 1986
Mouse, teratogenicity	Foetal skeleton abnormalities	0.5	Khera et al., 1982
Mouse, reproduction toxicity	Postnatal mortality	0.375	Khera et al., 1984
Swine, subchronic (85-100 days) (94-96 days) (naturally contaminated feed)	 Reduced growth Reduced growth and effects on liver and serum albumin	 0.04 0.06	 Bergsjø et al., 1992 Bergsjø et al., 1993

It was noted that the feed used in the studies with pigs was naturally contaminated, making it impossible to exclude the presence of other toxins, which may have contributed to the observed effects. Therefore these studies could not be used to derive a TDI.

It was decided to use the NOAEL (0.1 mg/kg b.w./day) of the chronic dietary study with mice with the application of an uncertainty factor of 100. A temporary TDI (tTDI) of 1 µg/kg b.w. was derived.

This tTDI-value would also protect against the other subchronic and reproductive effects listed in the Table as well as the acute vomiting effect of DON.

The tTDI is made temporary because it is noted that DON belongs to the group of several trichothecenes with a common basic chemical structure which are produced by *Fusarium* fungi (e.g.T-2 toxin, HT-2 toxin, nivalenol). According to present knowledge they also share common mechanisms of toxic action. Once the other most important trichothecenes have been evaluated, the Committee will consider the combined total exposure to trichothecenes and whether a group TDI should be assigned.

The intake of DON from cereals and beer has been estimated in the Nordic countries (Eriksen and Alexander, 1998) and from cereals in The Netherlands (Pieters et al., 1999) and was found to be in the order of the tTDI.

Needs for future studies

Studies are needed to cover the trichothecene group as a whole, to confirm that there are no neurotoxic effects at doses below those causing effects on growth and body weight, focussing on the known target for trichothecenes, the CNS serotonergic system.

The role of 3-acetyl-DON, which is prevalent in Europe, and 15 acetyl-DON, which is prevalent in North America, should be investigated. These toxins are often present at levels of 10-20% of DON, and differ only with an acetyl group. Research should be carried out to investigate the rate and degree these toxins are metabolised to DON to clarify if they can be assessed together with DON.

Comparative toxicity and toxicokinetic studies in rodents and pigs might allow insight in species differences.

There is a need for more accurate information on the exposure to DON (and other trichothecenes).

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