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OPINION

OF THE

SCIENTIFIC COMMITTEE ON FOOD

ON

FUSARIUM TOXINS

PART 5: T-2 TOXIN AND HT-2 TOXIN

adopted on 30 May 2001

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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 (SCF 1999), zearalenone (SCF 2000a), fumonisin B1 (SCF 2000b), nivalenol (SCF 2000c), and T-2 toxin and HT-2 toxin.

The present evaluation deals only with T-2 toxin and HT-2 toxin; 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). It does not address all the terms of reference outlined above for fusarium toxins in general. The Committee will address the general aspects when all the individual toxins have been considered.

T-2 and HT-2 toxin

Description

T-2 toxin and HT-2 toxin are mycotoxins of the group trichothecenes type A produced by fungi of the *Fusarium* genus, *i.e. Fusarium acuminatum, Fusarium poae* and *Fusarium sporotrichioides,* which are commonly found in various cereal crops (wheat, maize, barley, oats, and rye) and processed grains (malt, beer and bread). T-2-and HT-2 toxin often occur together in infected cereals. 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

T-2 toxin (fusariotoxin T2, insariotoxin, NSC 138780, T-2 mycotoxin, toxin T-2, T2-toxin, T-2 trichothecene): 12,13-Epoxytrichothec-9-ene-3,4,8,15-tetraol 4,15-diacetate 8-(3-methylbutanoate, $C_{24}H_{34}O_9$, MW: 466.58, CAS no.:21259-20-1.

HT-2 toxin (Toxin HT 2): 12,13-epoxytrichothec-9-α,4-β,8-α,15-tetraol 15 acetate 8-isovalerate, $C_{22}H_{32}O_8$, MW 424.54, CAS no.: 26934-87-2

T-2 toxin is rapidly metabolised to HT-2 toxin, which is a major metabolite *in vivo* and therefore, a common risk assessment for T-2 toxin and HT-2 toxin appears reasonable (Eriksen and Alexander, 1998).

The trichothecenes are in general very stable compounds, both during storage/milling and the processing/cooking of food, and they do not degrade at high temperatures (Eriksen and Alexander, 1998).

Biochemical aspects – cellular changes and potential modes of action

Effects on DNA and RNA-synthesis

T-2 toxin could inhibit synthesis of DNA and RNA both *in vivo* (0.75 mg/kg bw single or multiple doses) and *in vitro* (> 0.1-1 ng/ml) (Rosenstein and Lafarge-Frayssinet, 1983; reviewed in WHO, 1990).

Effects on protein synthesis

T-2 toxin had a strong affinity for the 60 S ribosomal subunit, and inhibited the activity of peptidyl transferase, and consequently also protein synthesis in the initiation phase (Beasley, 1989) (see also section on Apoptosis). T-2 toxin inhibited protein synthesis both *in vitro* (0.01 ng/ml in suspensions of rat hepatocytes gave 75 % inhibition) and *in vivo* (reviewed in WHO, 1990). *In vivo* inhibition of synthesis of proteins has been demonstrated in cells from bone marrow, spleen and thymus (0.75 mg/kg bw single dose *i.p.* in mice) (Rosenstein and

Lafarge-Frayssinet, 1983; Feinberg and McLaughlin, 1989; Thompson and Wannemachter, 1990; WHO 1990).

Effects on membranes and lipid peroxidation

T-2 toxin affected the permeability of cell membranes *in vitro* at concentrations of 0.4 pg/ml (Bunner and Morris, 1988), and caused changes in the phospholipid turnover in bovine platelets (Grandoni *et al*., 1992) and haemolysis of erythrocytes *in vitro* (Rizzo *et al*., 1992). One single oral dose of 3.6 mg/kg bw increased lipid peroxides in the liver of rats (Rizzo *et al*., 1994). Ascorbic acid, α-tocopherol and selenium (Tutelyan *et al*., 1990; Rizzo *et al*., 1992, 1994) as well as glutathione precursors (Fricke and Jorge, 1991) have a protective effect against lipid peroxidation induced by T-2 toxin.

Apoptosis

T-2 toxin caused apoptosis both *in vitro:* HL-60 cells, 10 ng/ml (Ueno *et al*., 1995), Raw 264.7 cells, 10 ng/ml, (Yang *et al*., 2000) and Jurkat cells, 10 µM (also: HT-2 toxin, T-2 triol, T-2 tetraol) (Shifrin and Anderson, 1999), and *in vivo* (10 mg/ kg bw) in thymic and splenic lymphocytes as well as other tissues of mice e.g bone marrow and intestinal epithelial crypt cells of mice (Li *et al*., 1997; Shinozuka *et al*., 1998).

The ability of T-2 toxin and different metabolites to induce apoptosis of the thymus were studied in four-week old female BALB/c mice (Islam *et al*., 1998). The mice were injected intravenously once with 1.56 mg toxin/ kg bw. Apoptosis and decreased cellularity in the thymus were recorded at different time points. Apoptosis occurred 1 hr after injection of 3-OH-T-2 and reached a maximum after 12 hrs whereas thymic atrophy was maximal after 4 days. The rank order of the potency to induce thymic apoptosis was T-2 = 3-OH-T-2 > HT-2 = 3-OH-HT-2 > neosolaniol =T-2 tetraol = vehicle control.

The mechanism of induction of apoptosis by trichothecenes is not clear. However, it has been found that T-2 toxin as well as metabolites such as T-2 triol and T-2 tetraol can activate the stress-activated kinases c-Jun N-terminal kinase 1 (JNK1) and/or p38MAPK (SAPK2). It has been suggested that trichothecenes and other peptidyl transferase inhibitors trigger a ribotoxic stress response causing the activation of MAP kinases (Shifrin and Anderson, 1999). Such activation may signal both cell survival or induce cell death in various cell types depending on the conditions, e.g. length of signal, and concurrent protein inhibition seem to favour cell death. The pathway leading from the activation of these kinases to the activation of caspases and apoptosis is not clear. It has also been reported that T-2 and other trichothecenes can activate other MAP kinases, i.e. extracellular signal regulated protein kinase 1 and 2 (ERK1/2), which usually mediates cell proliferation (Yang *et al*., 2000). The balance between ERK1/2 pathway and the stress activated JNK/ p38 MAP kinase pathway has been proposed to be fundamental for whether cell survival or apoptosis occurs. It appears that different metabolites of T-2 toxin as well as other trichothecenes differ in their ability to inhibit protein synthesis and activate the MAP kinases and induce apoptosis (Shifrin and Anderson, 1999; Yang *et al*., 2000). This apparently also differs between cell types. The outcome of such processes in different tissues and cells in animals exposed *in vivo* to T-2/HT-2 toxin where several metabolites coexist is difficult to predict.

Other effects

T-2 toxin also inhibited the mitochondrial electron transport system in yeast by inhibiting succinic dehydrogenase (Khachatourians, 1990). T-2 toxin inhibited gap-junctional intercellular communication in Chinese Hamster V79 cells (IARC, 1993).

Haematopoietic progenitor cells appears to be a sensitive target for T-2/HT-2 toxin toxicity both *in vivo* and *in vitro* (Parent-Massin and Parchment, 1998). However, no mechanisms of action have been established except that it is likely that those described above may operate.

Toxicokinetics

No human data on the kinetics of T-2 or HT-2 toxins are available.

T-2 toxin is rapidly absorbed after ingestion in most animal species and it is distributed in the organism with little or no accumulation in any specific organs. Maximum plasma concentrations occurred after about 30 minutes in rodents (reviewed in WHO 1990; IARC, 1993). Four hours after intravenous administration of radioactive labelled T-2 toxin to pigs, 15-24 % of the radioactivity given was found in the gastrointestinal tract and 4.7-5.2 % in the remaining tissues, mainly muscle and liver (Corley *et al*., 1986).

The plasma half-life for T-2 toxin is less than 20 minutes. T-2 toxin is rapidly metabolised by deacetylation, hydroxylation, glucuronide conjugation and de-epoxidation. The main biotransformation pathway is deacetylation of the C-4 acetyl group of T-2 toxin, which leads to HT-2 toxin (the sole metabolite of T-2 toxin with isolated microsomes from liver, kidney and spleen of various animals). This reaction is catalysed by a non-specific carboxyesterase in several tissues, mainly in the liver, but also in blood plasma (Johnsen *et al*., 1988), intestines (25% of T-2 is converted to HT-2 in the isolated intestinal loop assay) (Conrady-Lorck *et al*., 1989). HT-2 toxin may be further deacetylated, hydroxylated and conjugated by various metabolic pathways to 3'hydroxy HT-2, T-2 triol, 3'hydroxy T-2 triol, 4-deacetylneosolaniol which is converted to T-2 tetraol, and glucuronide conjugates of these. T-2 toxin can also undergo direct hydroxylation to 3'hydroxy T-2. These metabolites have been observed in several rodent species and pigs. Also cells *in vitro* such as lymphoid cells metabolise T-2 toxin (WHO, 1990; IARC, 1993).

In cynomolgus monkeys exposed intravenously to one dose of radiolabelled T-2 toxin more than 22% were found as metabolites in plasma after 5 minutes. After 2 hours 25 % of the amount in plasma was found as T-2, and only 8 % after 24 hours. Less than 2 % of the total plasma amount was found as HT-2 toxin in plasma at all time points, presumably due to rapid turnover to other metabolites. Major metabolites in plasma were T-2 tetraol and 3'hydroxy T-2. Major metabolites in urine was T-2 tetraol, 3'hydroxy HT-2 and 3'hydroxy-T-2. Metabolites could be observed in urine up to five days after exposure (Naseem *et al*., 1995). The amount of conjugates, e.g. glucuronide derivatives, was not determined.

In pigs, 63% of the total T-2-metabolites in urine and 77% in bile were glucuronic acid conjugates (Corley *et al*., 1985). In rodents, liver is the major site of biotransformation of T-2 toxin with biliary excretion as the major route (reviewed in IARC, 1993; Matsumoto *et al*., 1978). T-2 and 3-hydroxylated metabolites in the range from 10-160 μg/kg have been detected

in milk from cows intubated daily with 180 mg T-2 (Yoshizawa, *et al*., 1981). T-2 toxin is rapidly metabolised and secreted and no significant accumulation of T-2 is observed in the species tested (e.g. pig, cattle, dog, guinea pig).

HT-2 toxin, 0.06 mg/kg bw, was given in a single oral dose by gavage to mini-piglets and serum concentrations of HT-2 toxin were determined after 0.5, 1, 2 and 4 hours. HT-2 toxin appeared rapidly in serum and reached maximum concentration (1.3 µg/l) after 1 hour. At four hours the concentration was 0.8 µg/l (Bernhoft *et al*., 2000).

Since HT-2 toxin is a major metabolite of T-2 toxin (as was shown in *in vitro* studies with liver S-9 fraction from pigs, in which more than 80 % of T-2 toxin was converted to HT-2 toxin and metabolites thereof (Kabilka, 1985, see also above)) the same authors (Bernhoft *et al*., 2000) determined HT-2 toxin in serum of mini-piglets following a single oral dose of 0.06 µg T-2 toxin /kg bw HT-2 toxin appeared in serum at time points 0.5, 1, 2 and 4 hours. The serum T-2 toxin- and HT-2 toxin concentrations at 1 hour were approximately 1.3 and 0.6 µg/l, respectively. It is therefore reasonable to assume that HT-2 will follow the same metabolic pathway as T2 toxin (Eriksen and Alexander, 1998).

Toxicity

Acute and subacute toxicity

Acute effects of T-2 toxin occur after oral exposure to 0.06 - 10 mg/kg bw in various species. The effects observed include non-specific symptoms like weight loss, feed refusal, dermatitis, vomiting (cats, dogs, pigs and ducklings), diarrhoea, haemorrhages and necrosis of the epithelium of stomach and intestine, bone marrow, spleen, testis and ovary. Cats appear to be particularly susceptible to T-2 toxin (see also section on Haematotoxicity) (WHO, 1990; IARC, 1993; Rafai *et al*., 1995a; Eriksen and Alexander, 1997). A primary target of toxicity following one or more doses in studies on acute toxicity is haematopoetic tissue i.a. in the bone marrow (for further description see section on Haematotoxicity). Toxicity of the gastrointestinal epithelium following acute exposure is also a systemic effect, as it is not only observed following oral - but also parenteral exposure (DeNicola *et al*., 1978; Pang *et al*., 1987)

Acute disturbances in the circulatory system (hypotension and arrhythmia), which may result from the general pathophysiological responses to T-2 toxin including a central effect on blood pressure and catecholamine elevation, have been reported in pigs and rats (reviewed in Bubien *et al*., 1989 and WHO 1990). General arteriosclerosis and hypertension as delayed sequela of repeated exposure to T-2 toxin have been reported (Wilson *et al*., 1982). In a series of studies on rats exposed to a limited course of T-2 toxin doses caused thickening of coronary arteries including myocardial changes. Four *i.p.* injections of 3 mg T-2 toxin/ kg bw on alternate days gave vacuolisation and swelling of endothelial cells, basement membrane changes and enlarged medial smooth muscle cells. Upon topical application of T-2 toxin (1-20 µg) to rat skin dilated microvessels packed with erythrocytes and an increased number of degranulated mast cells were observed (Yarom *et al*., 1986, 1987a,b).

T-2 toxin has high acute toxicity, with acute oral LD_{50} values in rodents in the range of 5-10 mg/kg bw. Newborn animals seem to be more sensitive than older animals. T-2 toxin and HT-2 toxin have *i.p*. LD_{50} in the same dose range in mice (5-10 mg/kg bw). No oral LD_{50} has been established for HT-2 toxin.

Subchronic toxicity

Young pigs were fed 1 to 16 mg purified T-2 toxin/kg feed (equivalent to 0.04 to 0.64 mg/kg bw, source of toxin not given) for 8 weeks (Weaver et. al., 1978). No haematological effects were seen, while reduced body weight gain was observed at 1 mg/kg feed. The pigs refused a diet containing 16 mg T-2/kg, but not a diet containing 10-12 mg T-2/kg.

Twelve-week-old pigs fed 0.4 to 3.3 mg purified T-2/kg feed (equivalent to 0.016 to 0.132 mg/kg bw/day) for 5 weeks had no effects on haematocrit, red blood cell count, cell volume, or haemoglobin concentration. There was a non-significant tendency towards a lowered feed consumption in the exposed groups (Friend *et al*., 1992).

Mini-piglets were dosed with 0.012 or 0.06 mg T-2 or HT-2 toxin/ kg bw by gavage once daily from age 1 week to 8 weeks (Bernhoft *et al*., 2000). The piglets were vaccinated with tetanus toxoid, influenza-, herpes- and reo virus at ages 3 and 6 weeks. No effects caused by the treatment on clinical health, weight gain, haematology and antibody responses to vaccination were observed. Neither were any treatment-related changes found by macroscopical and histopathological examination at the end of the study.

In a study with seven weeks old pigs given feed containing 0.5, 1.0, 2.0, 3.0, 4.0, 10.0 or 15.0 mg pure T-2 toxin/kg feed for 3 weeks, lowered levels of glucose, inorganic phosphorus and magnesium were observed at doses from 1.0 mg/ kg feed (equivalent to 0.06 mg/kg bw). Except for a 10 % reduction in feed intake no effects on weight gain or other parameters were found among pigs fed 0.5 mg/kg feed (equivalent to 0.03 mg/kg bw) (Rafai *et al*., 1995a).

In a another study that was reported by Rafai and co-workers (Rafai *et al*., 1995b), on seven-week-old pigs exposed to T-2 toxin in the feed for three weeks immunotoxicity was observed at doses of 0.03 and 0.06 mg/ kg bw (see section on Immunotoxicity for further description).

Male rhesus monkeys (7 animals) were given 0. 1 mg T-2 toxin/kg bw by gavage daily for 4-5 weeks. There were no controls. Blood was drawn before the start of the experiment. At an early stage of treatment 3 animals developed complication notably vomiting, haemorrhage, respiratory infection, and died. In the remaining animals effects observed were a 40% reduction in leukocyte counts, reduction in phagocytosis of *E. coli* and a reduction in B-cells and T-cell lymphocytes (Jagadeesan *et al*., 1982).

Chronic toxicity and carcinogenicity

In mice fed 10 and 15 mg T-2/kg feed (equivalent to 1.5 and 2.25 mg/ kg bw) for 12 months, lesions (hypoplasia, hyperkeratosis and acanthosis) in the oesophageal region were observed. Such changes in the squamous epithelium were observed after 13 weeks of feeding and during the rest of the experimental period. The lesions were reversible and most had subsided 3 months after the exposure was terminated (Ohtsubo and Saito, 1977). In Wistar rats given 5 to

15 mg T-2/kg feed for 4 weeks, lesions similar to those observed in mice in the epithelium of the oesophageal region of the stomach were observed in rats receiving both 10 and 15 mg T-2 toxin/kg feed. These lesions were negligible in rats fed 5 mg/kg feed (equivalent to approximately 0.5 mg/ kg bw) (Ohtsubo and Saito, 1977). No adverse effect was observed in Holtzman albino rats fed 10 mg T-2/kg feed for 8 months (Marasas *et al*., 1969).

Mice (50 males and 50 females per group) were given feed containing 0, 1.5 or 3,0 mg purified T-2/kg feed (approx. 0.23 or 0,45 mg/kg bw/day) for 16 months. In males the incidence of pulmonary adenomas was 10% (4 animals) in controls, 15% (7 animals) for the low dose and 23.3 % (11 animals) for the high dose. Two controls and three high-dose males had pulmonary adenocarcinomas. In addition, the incidence of hepatocellular adenomas in high-dose male mice was increased compared to controls (control 3 animals (7%), low dose 3 (6%) and high dose 10 (21 %)). Significant differences in incidences of pulmonary and hepatocellular adenomas were found between males in the high dose and control groups ($p < 0.05$). A dose-related increase was observed in epithelial hyperplasia in the forestomach with a statistically significant difference between all treatment levels in both male and female mice. A dose related increase in heart weight was found in males, statistically significant for the highest exposed group, but not female CD-1 mice. No effects were found in other organs, haematological parameters or in T-dependent humoral immunity tests (response towards SRBC) (Schiefer *et al*., 1987).

The Committee was aware of a study in mice (Yang and Xia, 1988 cit. IARC, 1993) and two studies in rats (Li *et al*., 1992 (in Chinese) cit. IARC, 1993; Schoental *et al*., 1979) where forestomach papillomas (and forestomach and stomach epithelial hyperplasia or hyperkeratosis in the latter rat study) were observed, but could not use the information because of lack of information in the protocols.

T-2 toxin was negative in both initiator and promoter tests of skin tumour induction (reviewed in WHO, 1990; IARC 1993).

The Committee noted that IARC (1993) concluded that no data were available on the carcinogenicity to humans of toxins derived from *Fusarium sporotrichioides* and there is limited evidence in experimental animals for the carcinogenicity of T-2 toxin. The overall conclusion by IARC was that toxins derived from *Fusarium sporotrichioides* are not classifiable as to their carcinogenicity to humans (Group 3).

Genotoxicity

T-2 toxin was assayed in several *in vitro* and *in vivo* tests (see Haschek, 1989 and IARC, 1993 for reviews). T-2 toxin did not induce DNA damage in bacteria, gene mutations in *Salmonella typhimurium* or mitotic crossing-over or mitochondrial petite mutations in *Saccharomyces cerevisiae* with and without exogenous metabolic system. In cultured Chinese hamster V79 cells induction of gene mutations (0.1 µg T-2 toxin/ml) (activation by rat hepatocytes), and sister chromatid exchanges (activation by rat oesophageal epithelium), chromosomal aberrations and micronuclei (with and without exogenous metabolic system) were observed at very low concentrations. T-2 toxin slightly increased sex chromosome loss in *Drosophila melanogaster*, but the results for sex-linked recessive lethal mutations were inconclusive. T-2

induced chromosomal aberrations in cultured human lymphocytes in the absence of an exogenous metabolic system, but did not induce sister chromatid exchanges.

T-2 did not induce UDS in primary cultures of human gastric epithelial cells (Ji *et al*., 1994). In human fibroblasts, no UDS was observed after exposure to HT-2 toxin without metabolic activation. With liver S-9 activation the highest dose of HT-2 toxin caused UDS (see Haschek, 1989 and IARC, 1993 for review). T-2 toxin (0.005-0.5 µg/ml) and T-2 tetraol (0.1-10 µg/ ml), induced unscheduled DNA synthesis (UDS) in cultured human fibroblasts (Oldham *et al*., 1980). The UDS induction did not show any dose dependence, possibly due to concurrent cytotoxicity at all dose levels; moreover, at the highest doses protein and DNA synthesis are strongly inhibited.

Chinese hamsters treated in vivo with T-2 toxin had a slight increase in the frequency of chromosomal aberrations (1.70 mg/kg bw *i.p.*), but no increase of micronuclei (3.00 mg/kg bw *i.p.*). In mice 3 mg/kg bw *i.p.* (50% of LD_{50}) T-2 toxin caused a slight increase in single-strand breaks in spleen and thymus, but not in the liver (IARC, 1993). Bilgrami and co-workers (1995) studied the effect vitamin C (10 mg/ kg bw) on chromosomal changes in bone marrow cells of mice fed 0.10 ± 0.02 mg T-2 toxin /kg feed in the diet (equivalent to 0.015 mg/kg bw). T-2 toxin given for 12 weeks in the feed induced chromosomal abnormalities (mostly fragmentation, pulverisation and polyploidy, and no increase in chromosomal structural abnormalities was reported) and vitamin C reduced these effects. No dose response of T-2 toxin was investigated.

In vitro studies showing clastogenic effects have been carried out at concentrations between 0.0001 and 2.3 µg T-2 toxin/ml (IARC, 1993). Inhibition of protein synthesis has been found in rat hepatocytes at 0.00001 µg T-2 toxin/ml (see section on Effects on protein synthesis). DNA damage or clastogenicity were seen *in vivo* at doses of T-2 toxin also inducing general toxic effects.

Immunotoxicity

Various studies with T-2 toxin demonstrate effects on the production of antibodies, both in assays measuring T-dependent responses (e.g. response to sheep red blood cells, SRBC) and T-independent response (e.g. dinitrophenyl-bovine serum albumin or lipopolysaccharides, LPS) having effects on both humoral and cellular immune responses (Rafai and Tuboly, 1982; Taylor *et al*., 1989; WHO, 1990). Trichothecenes, as demonstrated for T-2 toxin and DON, can cause both suppression and stimulation of immunoglobulin production, depending on the doses and timing of exposure (WHO 1990, Pestka and Bondy 1994).

Rafai and co-workers (1995b) fed four groups of seven-week-old pigs weighing about 9 kg for three weeks with T-2 toxin. The concentrations were 0.5, 1.0, 2.0 or 3.0 mg/kg feed (prestarter), corresponding to average daily intakes of 0.38, 0.81, 1.24 or 1.43 mg, respectively. (Based on a corresponding experiment by the same authors (Rafai *et al*., 1995a) the mean weight during the experiment was calculated and it was found that the mean exposures would be equivalent to 0.03, 0.06, 0.1 and 0.13 mg T–2 toxin/kg bw, respectively.) At the first and fourth days of treatment the pigs were immunised with horse globulin. Blood

samples were drawn at days 7, 14 and 21. The two highest doses caused a significant reduction in the number of red blood cells at day 21. Leukocyte counts, particularly T-lymphocytes, were reduced at all doses. A reduced synthesis of antibodies towards horse globulin was seen at all doses and at all time points. Upon histopathological investigation of lymphoid organs at the end of the study the authors report dose dependent depletion of lymphoid elements in the thymus and spleen, but in the lymph nodes this was difficult to quantify. Even at the lowest dose (0.03 mg toxin/kg bw) the reduction in leukocyte count was 20 %, the response to horse globulin was reduced by 29 % and the blastogenic response of lymphocytes to PHA was reduced by 25 %. It should be noted that the feed intake was reduced by 10 % without any change in weight gain in this group (Rafai *et al*., 1995a). This could be due to potential confounding effects, as pair-fed controls were not used.

In another study with mini-piglets 0.012 and 0.06 mg T-2 – or HT-2 toxin / kg bw had no consistent imunotoxic effects (Bernhoft *et al*., 2000, see section on subchronic toxicity). In other studies in pigs no haematological changes were found with doses equivalent to 0.1 and 0.5 mg/kg bw (Weaver *et al*., 1978, Friend *et al*., 1992).

Thymus atrophy has been found in mice and rats after oral and intraperitoneal exposure. The lowest oral dose shown to induce thymus atrophy, particularly some subpopulations of thymocytes, in mice is 0.75 mg/kg bw (LOAEL = 0.75 mg/kg bw) (Smith *et al*., 1994).

Increased resistance towards bacteria in mice was observed after 0.5-3 mg T-2 toxin/kg bw (Corrier and Ziprin, 1986a, b, Corrier *et al*., 1987, Cooray and Jonsson, 1990). No effect on resistance towards bacteria were found after oral exposure to T-2 toxin to 0.75 mg/kg bw daily for a week prior to inoculation with bacteria, but an enhanced macrophage activity was observed (Atroshi *et al*., 1994, Cooray and Jonsson, 1990).

Effects of T-2 toxin on the human immune system

T-2 toxin reduced responses to mitogens in human lymphocytes *in vitro* (IC50: 0.9-1.3 nM). Combined with nivalenol an additive effect was seen whereas with DON a less than additive effect was seen (Thuvander *et al*., 1999). No *in vivo* data from humans with known exposures are available, but effects on lymphocytes were recorded in Russia in persons affected by the alimentary toxic aleukia, an epidemic where T-2 toxin is suspected to be the causative agent (see section on Human studies) (Taylor *et al*., 1989, WHO 1990).

Haematotoxicity

The effects of T-2- and HT-2 toxin on red cell-, leukocyte- and platelet progenitor cells from mice, rats and humans *in vitro* have been investigated with respect to effects on proliferation, differentiation and cytotoxicity. Such cells seem to be sensitive to toxic effects of both T-2 and HT-2 toxins as inhibitory effects on both parameters appear to occur in concentrations between 10^{-7} and 10^{-10}M (0.05-50 ng/ml) (Dugyala *et al*., 1994; Lautraite *et al*., 1995,1996; Rio *et al*., 1997). In foetal liver haematopoietic precursor cells from mice T-2 toxin (10^{-7} M, 50 ng/ml) resulted in highly selective and nearly complete toxicity to a subpopulation of $CD45R^{+}$ B-lymphocytic cells (Holladay *et al*., 1995).

Human circulating blood cells *in vitro* are less sensitive to T-2- and HT-2 toxin than other progenitor blood cells (see section on effects on membranes and lipid peroxidation).

Haematopoietic tissue *in vivo* is a target of toxicity in several animal species such as mice, rats, cats, rabbits and guinea-pigs following acute exposure to one ore more doses of T-2 – or HT-2 toxin (WHO, 1990).

A single intramuscular injection of 0.65 mg T-2/kg bw (LD_{20}) in cynomolgus monkeys caused transient leukocytosis, prolongation of prothrombin time and a decrease in coagulation factors (Cosgriff *et al*., 1986).

Adult rhesus monkeys weighing 2-3 kg were given T-2 toxin semi-purified by extractions from *Fusarium incarnatum* grown on rice (Rukmini *et al*., 1980). The final extract contained only T-2 toxin and no other trichothecenes. In the control group there was three females and three males and the treated group consisted of three males and two females. The treated group was given 1 mg T-2 toxin/ kg bw/day for four days in milk by stomach tube. The males started to vomit on the second day and became weak and the daily dose was reduced to 0.5 mg /kg bw from day 5-15. The male monkeys had skin haemorrhages and died from respiratory failure and lung congestion. Severe leukopenia as well as a decrease in haemoglobin concentration and platelet count were observed in all treated animals by the end of week one and in the surviving females at day 15. After 30 days of recovery the two females and two additional male monkeys were given 0.1mg/kg bw/day for 15 days. Leukopenia developed slowly in this group and was marked at day 15; also the haemoglobin concentration was reduced. Autopsies and histopathology were not reported for the low dose experiment.

Four groups of cats with 4-6 animals in each group were exposed to T-2 toxin from *F. sporotrichioides* No. 921 a strain associated with a fatal outbreak of alimentary toxic aleukia (ATA) (Lutsky *et al*., 1978). A crude extract of T-2 toxin, a crude extract free of T-2 toxin and purified T-2 toxin were prepared. The dissolved toxin was administered in gelatine capsules on alternate days. Groups of six and four cats received 0.08 or 0.1 mg purified T-2 toxin/kg bw, respectively. And groups of four and six cats received 0.06 or 0.076 mg T-2 toxin in crude extract/ kg bw, respectively. Crude extract from which T-2 toxin had been removed was given to two cats and diethylene glycol-ethanol-water solution was given to two cats, the latter two groups serving as controls. Following 16 doses the controls groups were healthy. Except for one cat receiving 0.076 mg T-2 toxin from crude extract all the other T-2 toxin exposed cats died between day 6 and 40. The mean survival time was dose dependent and varied from

13.5 to 34.5 days in the highest and lowest dose. The signs included emesis, anorexia, bloody diarrhoea, ataxia and weight loss. The lesions included multiple haemorrhages in the skin, intestinal tract, lymph nodes and heart, necrosis of gastrointestinal epithelium and decreased cellularity of bone marrow, lymph nodes and spleen. A slight leukocytosis occurred early followed by pancytopenia with a marked leukopenia after two weeks.

Cats appear to be extremely susceptible to T-2 toxin induced haematotoxicity and general toxicity with high lethality in comparison with other experimental species. The reason for this could be that cats in contrast to several other species, e.g. rodents, monkeys, pigs and humans, lack the ability to form glucuronide conjugates particularly of planar phenolic compounds (Timbrell, 1991; Court and Greenblatt, 1997 a,b). UDP-glucuronosyltransferase (UGT) 1A6 is a pseudogene in the cat and there is a reduced diversity of expressed hepatic UGT1A isoforms (Court and Greenblatt, 2000). Glucuronidation of various hydroxylated T-2 and HT-2 metabolites followed by urinary and biliary excretion are important detoxification mechanisms of these toxins in several other species, e.g. rats, mice, pigs. The biotransformation of T-2 toxin in cats has, however, not been examined.

In guinea-pigs 0.9 mg T-2 toxin/ kg bw and day for 27 days caused erythropenia, leukopenia, lymphopenia and reduced lymphocyte content of the bone marrow. More moderate changes were seen in animal given 0.5 mg T-2 toxin/ kg bw and day for 21 days followed by 0.75 mg T-2 toxin/ kg bw/day for 21 days (DeNicola *et al*., 1978).

Mice fed T-2 toxin in the diet, 20 mg/ dry diet (equivalent to about 3 mg/ kg bw) for 21 and 41 days showed hypoplastic lymphoid tissues, bone marrow and splenic red pulp resulting in anaemia (WHO, 1990). ^{59}Fe incorporation of circulating erythrocytes was strongly inhibited and granulocyte macrophage colony-forming cells in the bone marrow were transiently depleted by T-2 toxin in a single subcutaneous dose of 0.3 mg/kg (Faifer *et al*., 1992).

In mice treated with a single subcutaneous dose of 2 mg T-2 toxin/kg bw it was found following a potent initial inhibition, that spleen activity rapidly recovered while bone marrow remained significantly depressed for a much longer time (Velazco *et al*., 1996).

Rafai and co-workers (1995b) fed four groups of seven-week-old pigs weighing about 9 kg for three weeks with T-2 toxin. The pigs showed signs of haematotoxicity, see description in section on Immunotoxicity.

Pregnant mice were exposed to 1.2 or 1.5 mg T-2 toxin /kg bw by oral gavage on gestational days 14-17 and haematopoietic cells in foetal livers were investigated on gestational day 18. Subpopulations of cells with surface markers $CD44^{1o}$, $CD45^{1o}$, $CD45R^{+}$ were decreased. In adult mice exposed to 1.75 mg T-2 toxin /kg bw for four consecutive days bone marrow cellularity was reduced including cells with surface marker $CD45R^{+}$ but not cells with marker $CD44^{+}$ (Holladay *et al*., 1995).

Haematotoxic effects of T-2 toxin in humans

Alimentary toxic aleukia (ATA) was suggested to be related to the presence of *Fusarium* species in mouldy over-wintered grain. (See section on Effects in Humans)

Developmental toxicity studies

CD-1 mice were given 0, 0.5, 1.0, 2.0, 3.0, 3.5 and 4.0 mg T-2/kg bw orally on day 9 of gestation. Maternal mortality was observed in mice given the two highest doses. No foetuses were produced in the group receiving 4.0 mg/kg bw and a significant reduction in number of foetuses was seen in the group receiving 3.5 mg/kg bw. In mice given 3.0 mg/kg bw, a small increase in the incidence of skeletal deformities in foetuses was observed. No effect was observed in the group receiving 2.0 mg/kg bw or less. Mice given 3.0 mg/kg bw on day 6, 7, 8, 10, 11 or 12 of gestation showed less foetal mortality than mice given the same dose on day 9, but reduced litter size was observed also in the mice treated on day 7 (Rousseux and Schiefer, 1987).

In CD-1 mice fed 0, 1.5 or 3.0 mg T-2 /kg feed (equivalent to 0.23 or 0.45 mg/kg bw/day) for two generations neither embryotoxicity nor foetotoxicity was observed. Only a small transient reduction in growth of second-generation offspring was observed in the high dose group (Rousseaux *et al*., 1986).

Mice were given 0, 0.5, 1.0 and 1.5 mg pure T-2 toxin /kg bw/day intraperitoneally on days 7-11 of gestation. The two highest doses lead to maternal mortality and increased embryo-mortality. In litters surviving from these two dose levels, reduced foetal weight, missing tails, deformities in the limbs, open eyes and retarded jaw development were observed. No effects were observed in mice given 0.5 mg/kg bw (Stanford *et al*., 1975).

Wag rats were given T-2 toxin in the diet (2.5 and 10 mg/kg feed, equivalent to 0.1 or 0.4 mg/kg bw/day) or by daily *i.p*. injections (0.1, 0.2 or 0.4 mg/kg bw) on days 14-20 of gestation. No embryotoxic or teratogenic effects were observed at doses not leading to maternal toxicity. Foetal thymus atrophy, which was reversed after one week, was observed without any toxic effects on litter size, foetal resorption, maternal or foetal body weight, maternal liver or uterine weight. Two hours after injection of radiolabelled T-2 toxin significant concentrations of radioactivity were measured in the foetus. Thus, T-2 toxin or metabolites thereof cross the placenta in rats. (Lafarge-Frayssinet *et al*., 1990; Bertin *et al*., 1978 cited in IARC, 1993).

Pregnant mice were given an oral dose of 3 mg T-2 toxin/kg bw on day 11 of gestation. The embryos were examined after 24 hours and apoptosis was observed in some layers of the central nervous system, caudal sclerotomic segment, caudal region of the tongue, trachea and facial mesenchyma (Ishigami *et al*., 1999).

Effects on the nervous system

Exposure to T-2 changed the levels of neurotransmitters (dopamine, serotonin, tryptophan, 5-hydroxy-3 indoleacetic acid, 3,4-dihydroxyphenylacetic acid) in rat brain following dietary exposure to 2-21 mg T-2 toxin /kg bw/day. The LOAEL was 2 mg /kg bw/day (reviewed in WHO, 1990; MacDonald *et al*., 1988; Wang *et al*., 1993a). The level of 5-hydroxy-3

indoleacetic acid was increased in rats given 2.5 and 10 ppm T-2 in the feed (equivalent to 0.1 and 0.4 mg/kg bw/day) (Wang *et al*., 1993a).

In various behavioural tests rats given a single oral dose of 2.0 mg/kg bw showed reduced motor activity and performance in the passive avoidance test. No effect (NOAEL) was observed in rats receiving 0.4 mg/kg bw (Sirkka *et al*., 1992).

Dermal effects

T-2 toxin produces oedema, intradermal haemorrhage and necrosis of the skin. Guinea pig is the most sensitive species. The effect on skin has been used as a biological assay for detection of trichothecenes. T-2 can be detected at 0.2 μg with a skin necrosis assay. The minimum effective amount needed to elicit irritation is much less. The mechanism for skin toxicity has not been established (reviewed in WHO, 1990).

Effects in humans

Alimentary toxic aleukia (ATA) that occurred in the USSR in the period 1941-47 was suggested to be related to the presence of *Fusarium* species in mouldy over-wintered grain. An association to *F. poae* and *F. sporotrichioides*, which in later fungal cultures have been found to produce several trichothecenes including T-2 – and HT-2 toxin, was established. Extractions of the suspected wheat were also tested on the skin of animals, and showed toxic dermal effects. The most severe outbreak of the disease was in 1944, but outbreaks have also been reported in 1952, 1953, and 1955 particularly in people consuming over-wintered wheat. Clinical symptoms include vomiting, abdominal pain and diarrhoea followed by leukopenia, bleeding from the nose and throat, depletion of the bone marrow and fever. Depending on severity necrotic lesions in the oral cavity, oesophagus and stomach may occur and the lethality may be high. ATA in the second stage is characterised by leukopenia, haemorrhagic diathesis, granulopenia, bone marrow aplasia and sepsis. Although the symptoms characterising ATA resemble symptoms seen in cats and rodents exposed to crude extracts of infected wheat or T-2 toxin, no epidemiological studies have been reported to link trichothecenes to ATA (reviewed in Beardall and Miller, 1994; Joffe, 1974; WHO, 1990; IARC, 1993).

In an outbreak of toxicosis in a Chinese county 165 subjects consumed rice infected with *Fusarium heterosporum* and *F. Graminearum.* About 50 % of the persons consuming the rice fell ill with symptoms consisting of nausea, dizziness, vomiting, abdominal distension and pain, chills and diarrhoea. Samples of the suspected rice were analysed for T-2 toxin using an ELISA assay and a level of 180 - 420 μg/kg was found. Analysis for other toxins was not reported (Wang *et al*., 1993b). It has to be noted that these Fusarium species are not known to produce T-2 toxin, but are known to produce several other trichothecenes such as DON, nivalenol and acetyl-DON (Eriksen and Alexander, 1998).

Other outbreaks of trichothecene-related disease have also been reported, one in China 1984/85 and one in India in 1987. Each involved several hundred cases. Typical symptoms were throat irritation, nausea, vomiting, abdominal pain diarrhoea and some with blood in stools. In the first case DON and zearalenon were detected, but not T-2 toxin. In the outbreak in India DON, acetyl-DON, nivalenol and T-2 toxin were detected. No information on blood cells were reported and no fatalities occurred (WHO, 1990).

Evaluation and Conclusion

The general toxicity, haematotoxicity and immunotoxicity of T-2 toxin are considered to be the critical effects.

T-2 toxin potently inhibits synthesis of proteins and, at higher doses, DNA and RNA, both *in vitro* and *in vivo.*

There is limited evidence for tumourigenicity of T-2 toxin in experimental animals. It induced hepatocellular- and pulmonary adenomas in male mice. Several studies in mice and rats indicate that T-2 toxin cause toxicity and proliferative changes in the oesophagus- and forestomach epithelium.

Several conventional tests for genotoxicity *in vitro* and in rodents *in vivo,* in particular for clastogenic effects, were positive for T-2- and HT-2 toxin. These effects were observed primarily at concentrations also known to inhibit protein- and DNA-synthesis and produce cytotoxicity. Therefore, the Committee considered that the effects observed *in vitro* and *in vivo* were most likely secondary.

Only those studies considered to be of good quality and addressing relevant endpoints are summarised in Table I.

Table I. LOAELs and NOAELs of T-2 toxin

Study	**Critical Effect**	**LOAEL/NOAEL (mg/kg bw/day)**	**Reference**
Mouse, 16 months	Pulmonary adenomas Hepatocellular adenomas Forestomach epithelial hyperplasia	0.23 (NOAEL) 0.23 (NOAEL 0.23 (LOAEL)	Schiefer *et al*., 1987
Rat, 4 weeks	Forestomach epithelial hyperplasia	0.5 (NOAEL)	Ohtsubo and Saito, 1977
Mouse, 5 days	Thymus atrophy, decreased number of T- and B- cells	0.75 (LOAEL)	Smith *et al*., 1994
Pig, 3 weeks (subacute)	Reduced number of leukocytes and	0.03 (LOAEL)	Rafai *et al*., 1995b

	lymphocytes and reduced antibody production against horse globulin. Decrease in the lobule size of thymus and spleen (no regressive changes)		
Monkey (15 days of exposure by gavage)	Leukopenia	0.1 (LOAEL)	Rukmini *et al*., 1980
Mouse, CD-1, two generations. No dose showing effect.	Embryo- or foetotoxicity	0.45 (NOAEL)	Rousseaux *et al*., 1986
Rat, single dose	Neurotoxicity	0.4 (NOAEL)	Sirkka *et al*., 1992

The haematotoxicity and immunotoxicity of T-2 toxin in pigs in a short-term study are used as the basis for the safety assessment. Slight effects on immune parameters (Table I) and also a reduction in the feed intake by about 10 % were seen even at the lowest dose. Similar effects were not observed in other studies in pigs neither at this nor even at higher doses. Hence, it is likely that the LOAEL in the study by Rafai and coworkers (1995b) is close to the NOAEL.

Although the database is fairly extensive, there are deficiencies, e.g. study duration, pair feeding of control animals, comparative studies on metabolism and toxicokinetics. To account for this and the use of a LOAEL, presumably close to the NOAEL, an extra uncertainty factor of 5 was included, giving an overall uncertainty factor of 500. This would lead to a temporary TDI (t-TDI) of 0.06 µg T-2 toxin/kg bw. This t-TDI value would also protect against the other chronic, subchronic and reproductive effects listed in Table I.

The acute toxicity of T-2 toxin and HT-2 toxin are within the same range and T-2 toxin is rapidly metabolised to HT-2 toxin *in vivo*. The toxicity of T-2 toxin *in vivo* might well at least partly be attributed to HT-2 toxin. Hence, it is appropriate to set a combined t-TDI for the sum of T-2 toxin and HT-2 toxin.

The t-TDI is also temporary because it is noted that T-2 - and HT-2 toxin belong to the group of several trichothecenes with a common basic chemical structure which are produced by *Fusarium* fungi (e.g. DON and nivalenol). It is noted that according to present knowledge they also share common mechanisms of toxic action. The t-TDI is therefore established pending the evaluation of the group of trichothecenes as a whole.

This t-TDI is in-line with the TDI recently derived for T-2 and HT-2 toxin by JECFA (JECFA, 2001).

JECFA estimated the mean daily total intake of T-2 and HT-2 toxin in Europe to 0.017 µg/ kg bw on the basis of the critically evaluated levels in food and the GEMS/Food European regional diet (JECFA, 2001). In the Nordic countries the average daily total intake of T-2 and HT-2 toxin from cereals was estimated to be 0.13 µg/kg bw (Eriksen and Alexander, 1998). The Committee noted that much data on T-2 and HT-2 toxin in food is of low quality and

should not be used for intake estimations. Furthermore, the sensitivity of the analytical methods used was low in many cases, which can lead to an overestimation of intake depending on the method of for calculating intakes. Hence, comparison of intake estimates is very uncertain.

Needs for future studies

The haematotoxicity and immunotoxicity induced by T-2/HT-2 toxin should be investigated by the use of sensitive endpoints with respect to low dose-effects and time course in longer-term exposure studies where feed intake is controlled (e.g. 60 days in pigs).

Comparative studies on metabolism and toxicokinetics in cats, rodents and pigs might allow insight into species differences in toxicity. Studies of metabolism of T-2 toxin in humans using *in vitro* models would also be helpful.

There is a need for more adequate information on the exposure to T-2 toxin and HT-2 toxin (and other trichothecenes), i.e. occurrence in food commodities and intakes.

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