

ILSI Europe
Report Series

TRICHOHECENES WITH A SPECIAL FOCUS ON DON



Summary Report of a Workshop held in September 2003

Organised by the ILSI Europe Natural Toxin Task Force

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***TRICHOTHECENES WITH A SPECIAL
FOCUS ON DON***

**SUMMARY REPORT OF A WORKSHOP HELD IN SEPTEMBER 2003 IN DUBLIN, IRELAND
ORGANISED BY THE ILSI EUROPE NATURAL TOXIN TASK FORCE**

March 2004

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CONTENTS

INTRODUCTION	4
OBJECTIVES OF THE WORKSHOP	5
SESSION 1: AGRICULTURAL ASPECTS AND DISTRIBUTION PATTERNS OF INDIVIDUAL TRICHOHECENES	6
<i>Fusarium</i> taxonomy with relation to trichothecenes formation	6
Influence of agricultural practices and conditions on distribution patterns of <i>Fusarium</i> and trichothecenes in different crops	7
Crop resistance – <i>Fusarium</i> infection and trichothecenes formation	7
SESSION 2: HARVEST, STORAGE AND PROCESSING	9
Influence of harvest and storage conditions on trichothecenes levels in various cereals	9
Influence of processing on trichothecenes levels	9
SESSION 3: TOXICOLOGY	11
Cellular and molecular effects, including immunotoxic effects	11
Haematotoxicity and cardiovascular toxicity	12
Toxicity data relevant to hazard characterisation	13
Combined toxic effects	15
SESSION 4: SAMPLING AND ANALYTICAL METHODS	17
Sampling for trichothecenes analysis	17
State of the art of trichothecenes analysis	17
Rapid methods	18
Reference materials and validated methods	19
SESSION 5: ASSESSMENT OF EXPOSURE	21
Critical assessment of exposure – report from SCOOP project	21
Probabilistic exposure models	22
SESSION 6: RISK ASSESSMENT AND PREVENTION STRATEGIES	24
Risk assessment paradigm and its application for trichothecenes	24
Prevention strategies	26
SESSION 7: FINAL DISCUSSIONS AND CONCLUSIONS	28
Prevention	28
Sampling and analytical methods	29
Exposure assessment	30
Toxicology	31
Exposures above the TDI	32
ACKNOWLEDGEMENTS	33
ILSI EUROPE WORKSHOP PARTICIPANTS	34
REFERENCES	36

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INTRODUCTION

A variety of *Fusarium* fungi, which are common soil fungi, produce a number of mycotoxins of the class of trichothecenes (e.g. the type A trichothecenes: T-2 toxin (T-2) and HT-2 toxin (HT-2) and the type B trichothecenes: deoxynivalenol (DON), 3-acetyl DON, 15-acetyl DON, nivalenol (NIV) and 4-acetyl NIV (fusarenone X)) and some other toxins (zearalenone and fumonisins). They are commonly found on cereals grown in the temperate regions of Europe, America and Asia. The extent of infection is dependent on weather conditions, Good Agricultural Practice and storage conditions of cereal crops. Intoxications following consumption of foodstuffs contaminated with trichothecenes have occurred in both humans and animals with large numbers of people and livestock being affected.

The EC Scientific Committee on Food (SCF) has evaluated the *Fusarium* toxins DON (SCF 1999), NIV (SCF 2000) and T-2 and HT-2 (SCF 2001) and derived tolerable daily intakes (TDI) for humans. In addition, a group evaluation of T-2, HT-2, NIV and DON was attempted (SCF 2002). Some countries have already established legislative limits in cereals for DON, the most abundant trichothecene, and the European Commission has proposed EU regulatory limits for DON and T-2/HT-2 in various raw cereals and their refined products. These are currently being discussed among the member states.

The limits for DON proposed by the EU Commission (Codex Alimentarius Commission FAO/WHO, 2003) for various cereal and cereal products are as follows:

Unprocessed durum wheat and corn	2000 µg/kg
Other unprocessed cereals	1500 µg/kg
Wholemeal wheat flour, bran and pasta (dry)	750 µg/kg
Cleaned cereal for direct human consumption and all derived products	500 µg/kg
Maize based breakfast cereals and snacks	500 µg/kg
Cereal for infants and ingredients used in manufacture thereof	100 µg/kg

Unfavourable weather conditions in Western Europe have recently led to a high level of *Fusarium* infection in wheat and correspondingly high trichothecene contents. There is also an indication that during recent years the TDI for DON established by the SCF may occasionally have been exceeded, especially for children. To address all aspects concerning trichothecenes in food, a workshop on trichothecenes with a special focus on DON was organised by the ILSI Europe Natural Toxin Task Force. The workshop was convened in Dublin, Ireland on 10-12 September 2003. A number of experts representing different relevant disciplines from the area of mycotoxin research were invited to address specific scientific issues as the basis for a comprehensive risk assessment and recommendations for the development of preventive strategies. In addition, the workshop identified gaps in knowledge in order to encourage further research.

Dr. S. Page, World Health Organisation (CH), acted as the overall chairman with Dr. R. Battaglia, Swiss Quality Testing Services (CH), as the overall co-chairman. Both also acted as session chairs. In addition, Dr. M. Moss, University of Surrey (UK), and Prof. R. Kroes, University of Utrecht (NL), acted as session chairs. Dr. J. C. Larsen, Institute of Food Safety and Nutrition (DK), was the overall rapporteur with Prof. P. Ruckebauer, Institute for Agrobiotechnology (A), Dr. I. Perrin, Nestlé (CH), and Dr. J. Hunt, Kraft Foods (D) as session rapporteurs.

OBJECTIVES OF THE WORKSHOP

The aims of this two-day interactive meeting of experts were to:

- Review the current knowledge on trichothecenes with respect to occurrence (including mould growth, toxin formation, storage, effects of processing and prevention) and analytical methodologies (including sampling, surveillance and exposure assessments, toxicology and risk assessment), and;
- Identify additional issues that should be addressed (harmonisation of analytical methods and collection of data for exposure assessment, excursions above the ADI, acute toxicity, chronic low dose exposures) and gaps in knowledge, and;
- Suggest ways forward in providing advice to risk managers on problem formulation and reducing the uncertainties in the risk assessment and the development of prevention strategies.

SESSION 1: AGRICULTURAL ASPECTS AND DISTRIBUTION PATTERNS OF INDIVIDUAL TRICHOHECENES

Rapporteur: Prof. P. Ruckenbauer, Institute for Agrobiotechnology (A).

Fusarium taxonomy with relation to trichothecenes formation

Dr. M. Moss, University of Surrey (UK), explained that the *Fusarium* genus is important because many species are pathogens of important food and feed crops and several species produce toxic metabolites which find their way into the human food chain. Traditionally, species have been separated and described on the basis of morphology and cultural characteristics such as pigment production and the presence of microconidia. In recent years, multidisciplinary approaches and the rapid development in molecular biology have had a major impact on fungal taxonomy. Many recently described *Fusarium* species have been discovered by molecular tools or by the direct use of metabolite profiles in fungal taxonomy and identification. Applying a very simplified view of the structure of the genus Dr. Moss presented three groups of *Fusarium* species.

Those species of *Fusarium* which belong to the **Liseola** group are not known to produce trichothecenes, although *Fusarium verticillioides* is a very toxigenic species being one of the major producers of the fumonisins.

Fusarium sporotrichioides of the **Sporotrichiella** group is the most important producer of T-2 toxin. This species has no known teleomorph. It is essentially a saprophytic species and is especially associated with cereals left in the field after normal harvest. *Fusarium poae* is more widespread in Europe, and is an important producer of NIV.

Finally, the **Discolor** group contains the most important DON-producing species, *Fusarium graminearum* and *F. culmorum*. *F. graminearum* is a major cause of foot rot and head blight diseases of wheat and barley as well as ear and stalk rot of maize. Dr. Moss recognised three chemotypes in morphologically similar strains on the basis of the metabolites produced:

- Chemotype Ia produces DON and 3-acetyl DON (especially associated with the Far East),
- Chemotype Ib produces DON and 15-acetyl DON (especially associated with the New World) and;
- Chemotype II that produces NIV and 4-acetyl NIV (Fusarenone X).

Historically, two distinct groups of *Fusarium graminearum* were recognised. **Group 1** types are reported to be mainly soil-borne pathogens causing crown rot and foot rot of wheat, barley and oats. They are mainly from Australia, Africa and the Pacific Northwest of America. **Group 2** types seem to be typically associated with northern temperate regions of the northern hemisphere and are seed-borne pathogens causing ear rot of maize and head scab of wheat, oats and barley. These are now considered the true *F. graminearum*. The crown rot species is now called *F. pseudograminearum*.

The trichothecenes have significant phytotoxic activity, and are important factors in the initial colonisation of the living plant as well as the continuing invasion of plant tissue. They also have antimicrobial activity, and may be important regarding competition with other microorganisms, especially fungi.

Influence of agricultural practices and conditions on distribution patterns of Fusarium and trichothecenes in different crops

Dr. S. Edwards, Harper Adams University College (UK), explained the influence of agricultural practices and conditions on distribution patterns of *Fusarium* and trichothecenes in different crops. *Fusarium* head blight (FHB) of small grain cereals and ear rot in maize is caused by a number of *Fusarium* species and *Microdochium nivale*. Ear rot of maize in Europe can be divided into red ear rot, caused by trichothecene-producing *Fusarium* species in particular *F. graminearum* and pink ear rot, predominantly caused by non-trichothecene-producing *Fusarium* species such as *F. verticillioides*.

FHB pathogens are known to infect cereals primarily at anthesis (flowering), and the weather conditions during this period affect the incidence and severity of infection. These are warm dry springs, heavy rainfall at early flowering and warm humid conditions to allow spores to germinate and infect host tissues. *Fusarium* species have different optimum conditions for growth and infection; consequently, the dominant species present on any given cereal can differ between seasons and across regions. In recent years, *F. graminearum* has dominated in Western Europe, while in Scandinavia *F. avenaceum*, *F. culmorum* and *F. poae* dominate. In the United Kingdom, there is usually a trend towards *Microdochium nivale* in the North and *F. culmorum* in the South.

A number of agronomic practices are known to affect the infection, growth and toxin production of *Fusarium* species. These include crop rotation, varietal resistance, fungicide application and cultivation techniques. It is important to know what agronomic factors can affect mycotoxin contamination of grain so that "Good Agricultural Practice" can be formulated and used to advise growers in order to minimise mycotoxin contamination of grain. For instance, for FHB of wheat having maize as a previous crop is a risk factor, as are the resistance behaviour and the amount of crop debris remaining after harvest in the fields. Various fungicides have been shown to be active against FHB. Important differences in fungicide efficacy against *Fusarium* species have recently been demonstrated between triazoles, such as tebuconazole and metconazole, and azoxystrobin, but no significant differences were found in DON content after various other fungicide treatments. Dr. Edwards mentioned that the conclusions on the effects of agronomic factors on mycotoxin contamination of wheat were substantiated by results from a large project in which 300 United Kingdom wheat samples from the harvest of 2001 and 2002 were analysed for trichothecenes content.

Much less evidence is available for ear rot of maize or FHB of other small grain cereals in Europe. However, for maize much work has been done on insect control. Insect damage may result in the infection of tissues by *Fusarium* species. A positive relationship between degree of insect infestation and *Fusarium* mycotoxin contamination of maize has been found in North America, whereas results within Europe have been variable.

Crop resistance – Fusarium infection and trichothecenes formation

Dr. C. Snijders, Zelder Breeders and Seedmen (NL), gave an overview on the resistance in wheat to *Fusarium* head blight and trichothecenes formation. He stated that, in Western Europe, there is an increased use of high-yielding wheat varieties with greater than average susceptibility to *Fusarium* infection. *Fusarium* invasion can reduce kernel set and kernel weight causing a reduction in yield. It can also destroy the starch granules and cell walls and affect endosperm storage proteins resulting in a poor quality product. There is large variation in aggressiveness and

toxigenic potential among isolates of *F. graminearum* and *F. culmorum*. Greater colonisation of spikelets and grain is found for trichothecene-producing than for non-producing strains and DON production is an important aggressiveness factor in FHB. No evidence has been found for the occurrence of *F. culmorum* or *F. graminearum* adapted to different wheat genotypes.

There are morphological characteristics that help protect plants against infection in wheat. Tall plants without awns and plants with lax ears tend to have lower rates of natural infection than plants that are short, awned or have a high spikelet density.

Resistance to FHB can be characterised as consisting of two components: resistance to initial penetration and resistance to spreading of the pathogen in host tissue. In wheat *Fusarium* infection and DON content are correlated though, not consistently. Besides the resistance mechanisms that determine the severity of head blight, there may be other types of mechanism that influence kernel DON content, such as degradation or conjugation of DON and tolerance to DON. DON tolerance will result in an increased level of resistance and inherent prevention of trichothecenes accumulation.

Several investigators have tried to use sensitivity to DON as a rapid way to screen plants for resistance. It has been demonstrated that defence response genes are activated both in infected spikelets and in uncolonised parts of infected heads. It is not yet known which of the activated genes are important in disease resistance. Complete resistance has not been discovered.

The goal of a wheat-breeding programme is to develop superior genotypes. Breeding for phenotypic *Fusarium* resistance is difficult and time consuming. Although some good results were obtained for selection based on visual symptoms in wheat, the poor relationship between fungal biomass and visual symptoms, especially for the extremes on the head blight assessment scale, makes a well defined selection tool necessary. The use of molecular markers would increase the efficiency in a practical selection programme. Breeders need markers that can be used across populations, at a minimum for selections carrying the same source of resistance.

The policy of official variety list trials may affect the level of resistance of future varieties. In Germany candidate varieties must have a score of at least 5 on a 1-9 scale, where 1 is highly resistant. Candidate varieties with a score of 6 or higher are excluded from farmers' union trials, which will prevent marketing. For official national list testing in Germany candidate varieties are evaluated for head blight by testing them in a maize stubble field. This has resulted in the new varieties in the list being of the taller types.

Currently available and affordable methods of FHB control are only partially effective. There is an opportunity for biological control to become an important component of the integrated management of this disease. There are several approaches: spraying of antagonists at flowering time, treatment of infected seed with antagonists and combating inoculum production on crop residues by application of organisms that are specialised in degradation or colonisation. In effective biocontrol agents, anti-*Fusarium* proteins may be identified that could be of interest for transgenic approaches to combat *Fusarium* head blight.

SESSION 2: HARVEST, STORAGE AND PROCESSING

Rapporteur: Prof. P. Ruckebauer, Institute for Agrobiotechnology (A).

Influence of harvest and storage conditions on trichothecenes levels in various cereals

In his contribution on the influence of harvest and storage conditions on trichothecenes levels in various cereals Dr. R. Schrödter, Milupa (D), focused on the problem for food producers to obtain consignments of cereals with low levels of trichothecenes, in particular for production of foods for vulnerable groups of consumers like babies and infants. Generally, mycotoxins are formed by mould infection in the field, mainly caused by *Fusarium* species during flowering and during storage mainly by *Penicillium* and *Aspergillus* species. However, bad storage conditions with moisture levels of 20%-30% significantly increase the amounts of trichothecenes formed in *Fusarium* damaged wheat, for example, storage at 17-20% of remaining water has increased the amounts of trichothecenes in wheat within 6 weeks. Methods of control of infection during the vegetation period including crop selection, rotation and fungicide application, and harvesting and storage are currently being investigated.

The moisture levels during harvest, transport and storage of the grain are key factors. The various *Fusarium* species require at a minimum 17%-19% moisture content in the grain for growth. During harvest the moisture levels of grain in Europe may range from 15%-30% for wheat, oat, rye and barley with the highest moisture levels recorded in Northern Europe. For maize the range is 30%-40% moisture content. Therefore, grain should be harvested at minimised humidity levels and separated from root rot affected material. As *Fusarium* spores from soil are preferentially deposited on the stalk, the cutting height at harvest becomes important. A cutting height of 20 cm above the ground is recommended.

To avoid optimal growth conditions for fungi inside and outside the cereal kernels after harvest, the moisture content of grains should be at 14%-15% at 15°C for wheat, barley, rye and oat. If higher levels of humidity occur, appropriate drying in a reasonable time frame is necessary. The design of the drying facility is an often-ignored point of interest especially with regard to the build-up of wet spots in the bin.

Influence of processing on trichothecenes levels

Dr. S. Patel, RHM Technology (UK), on behalf of Dr. C.M. Hazel, addressed the influence of processing on trichothecenes levels. *Fusarium* mycotoxins occur frequently in cereal products such as wheat, maize, barley, oat and rye used as raw materials for food manufacture throughout Europe. Surveillance of retail food and drinks of cereal origin demonstrates that trichothecenes survive the various production processes employed. Trichothecenes are relatively heat stable with high water solubility. DON is stable at 120°C, moderately stable at 180°C and partially stable at 210°C. It is stable under weakly acidic conditions but is unstable in alkali conditions, such as encountered during tortilla preparation. A 72%-88% reduction in DON in contaminated maize has been recorded during this process.

Multiple studies have been conducted on the fate of mycotoxins during food production processes. The majority have focused on DON, some have included NIV and T2 toxin. Harvested cereals intended for food production are cleaned prior to milling; this removes impurities, such as straw and dust, but can also be used to take out broken or damaged grains. Grains heavily

infected by *Fusarium* become shrivelled and lower in weight than healthy grains and can be separated by physical means. It has been reported that grain cleaning has reduced DON levels by up to 74%. However, routine grain cleaning leads to, at best, a small (up to 20%) reduction in trichothecenes levels.

It is known that the extent of transmission into final food products depends on the pattern of *Fusarium* infection in the grains. Milling of cereal grains prior to producing the fractions required for food production falls into two categories, wet and dry milling. Wet milling is the major milling process used for maize and results in the production of food grade fractions such as maize starch and glucose syrups. Trichothecenes are water soluble compounds and are consequently found in the steep liquor fraction and gluten fractions, with low carry over into the starch and hence syrup fractions. For dry milled maize the highest levels of toxins occur in the germ and bran fractions with less in maize flour and grits – the streams that enter the food chain as breakfast cereals, snacks and polenta. After dry milling of wheat the highest concentrations of trichothecenes are found in the bran and wheat feed, with lower concentrations in white flour (50% of level in wheat). The most highly contaminated streams enter the animal food chain, although there is no evidence of significant trichothecenes transmission into animal products.

The processes used for baking bread and non-yeasted products (cakes/biscuits) vary considerably throughout the world (fermentation and baking conditions, time and temperatures, plus inclusion of additives to the dough mixture). The available data on the effects of yeast on DON levels are conflicting. In some studies, an increase in DON has been reported while other work suggested that DON is reduced by over 40% during dough fermentation. A limited number of studies have been conducted on other baking processes.

Pasta is produced from the semolina of ground Durum grains. During pasta boiling, DON is lost to the boiling water. In a discussion contribution, Italian researchers reported that cooked spaghetti retained on average only 18% of the original DON content of the durum grain. This was tested in samples within a range of DON concentration of between 0.3 and 13.2 µg/kg.

Extrusion is one process used in the production of food products such as breakfast cereal and snack products and typically involves high temperatures and pressures. The studies describing trichothecenes fate during extrusion again show varying results. DON is stable at the temperatures and pressures used in these processes, however, other inclusions within the extrusion mixture, such as the use of bisulphite, may reduce the final DON content.

Cereals, notably barley and to a lesser extent wheat, are the major raw materials of the brewing process. Although the different process steps have been reported to have variable effects on DON, the overall impression is that DON is stable during brewing.

Dr. Patel gave an overview of a recent survey by the UK Food Standards Agency in which 377 retail cereal samples were surveyed for a range of trichothecenes. Toxins were detected in 298 samples. DON was the most frequently occurring followed by NIV. The highest levels were found in breakfast cereals and snacks (largely maize-based samples), reflecting that typically the level of contamination is higher in maize than in smaller grain cereals. These results show that trichothecenes do survive milling, baking and extrusion processes.

SESSION 3: TOXICOLOGY

Rapporteur: Dr. I. Perrin, Nestlé (CH).

Cellular and molecular effects, including immunotoxic effects

Acute high dose toxicity of trichothecenes is characterised by “radiomimetic” effects such as diarrhoea, vomiting, leukocytosis, haemorrhage and circulatory shock and death, whereas chronic low dose toxicity is characterised by anorexia, reduced weight gain, diminished nutritional efficiency, neuroendocrine changes and immunologic effects. Basically, trichothecenes bind to eukaryotic ribosomes and inhibit protein synthesis by blocking translation and inhibiting the elongation of peptide chains. In his talk on cellular and molecular mechanisms for trichothecene-induced immune dysfunction, Professor J. Pestka, Michigan State University (USA), pointed out that the effect of trichothecenes on the immune system, paradoxically, can be both stimulatory and suppressive. This depends on the administered dose, the exposure frequency and timing of functional immune assays.

Low-dose trichothecenes exposure has immunostimulatory effects resulting in increased resistance to pathogens, elevated serum IgA levels and upregulation of the expression of many immune related genes such as those coding for cyclo-oxygenase-2 (COX-2), cytokines (Th1 and Th2), and chemokines. The induction of gene expression is under transcriptional and post-transcriptional (increased mRNA stability) control. Regarding immunostimulation, COX-2 induction is critical in driving the production of IL-6 by macrophages. IL-6 from macrophages and T cells is probably the crucial cytokine in mediating the differentiation of B cells to IgA producing plasma cells. In mice fed DON or nivalenol, IgA immune complexes deposit in the kidney and produce a glomerulo-nephritis resembling human disease.

In contrast, high-dose trichothecenes exposure severely injures actively dividing tissues including bone marrow, lymph nodes, spleen, thymus and intestinal mucosa resulting in immunosuppression evidenced by depression of circulating blood leukocytes, reduced serum IgM and IgG levels, decreased resistance to pathogens, inhibition of antibody responses to model antigens and impaired delayed type hypersensitivity responses. The suppressing effect on leukocyte function is linked to induction of apoptosis demonstrated *in vivo* and *in vitro* in macrophages, T cells and B cells. DON sequentially induces mitogen-activated protein kinases (MAPKs) phosphorylation (activation), transcription factor activation and COX-2 mRNA expression. The process in which compounds bind to ribosomes and rapidly activate MAPKs and apoptosis is known as “ribotoxic stress response”. The MAPKs, extracellular signal regulated protein kinases 1 and 2 (ERK 1 and 2) and p38 contribute to upregulation of inflammatory genes and cytokines. However, the effect on a given cytokine may differ between individual trichothecenes. Double-stranded RNA-(dsRNA)-activated protein kinase (PKR) and haematopoietic cell kinase (Hck) are upstream transducers of MAPKs and their activation contributes to leukocyte apoptosis via sequential activation of p38, p53, and caspase 3. *In vitro* studies have shown type D trichothecenes to be most potent in inducing apoptosis, and type B less than type A.

Professor Pestka hypothesised that when ERK and p38 are activated, expression of immune genes is favoured whereas when only p38 is activated an apoptotic response ensues. According to Professor Pestka several questions remain open: What are the molecular links between activation of kinases and effects on the ribosomes? Are the immune effects reversible or cumulative? Are the *in vitro* results consistent with *in vivo* data in animal models and with work on human cell cultures? What are the impacts of genetic polymorphism and of co-exposure to other mycotoxins or to infectious agents on the immune effects mediated by trichothecenes?

During the discussion, the question was raised whether the developing immune system would be particularly vulnerable. Could trichothecenes-induced immunomodulation in early life have an effect on the imprinting of the immune system, or could it elicit a deviation facilitating the development of allergies or autoimmune disorders? However, because the available *in vivo* studies in mice (effect of DON on IgA production) have all started at weaning this question could not be answered.

Haematotoxicity and cardiovascular toxicity

Haematopoietic cells have an important role in the immune system and immunomodulatory and haematotoxic effects of trichothecenes may represent a continuum. Haematotoxicity is a common major symptom seen in humans following consumption of food contaminated with trichothecenes. Professor D. Parent-Massin, Brest College of Higher Education (F), explained that the haematotoxicity in humans is characterised by thrombocytopenia and leukopenia as well as coagulation disorders and compromised resistance to infections. These disorders may consequently lead to septicaemia and massive haemorrhages eventually leading to death. The human data are derived from outbreaks of poisoning where several trichothecenes were involved. Possibly the best known incident is the outbreak of Alimentary Toxic Aleukia (ALA) in Russia from 1942-1947, where large number of fatalities were associated with over-wintered wheat and other small grains. The fungi responsible for the disease were identified as being *Fusarium poae* and *F. sporotrichioides*.

Subacute and subchronic ingestion of trichothecenes cause a decrease in circulating blood cells, frequently associated with bone marrow failure in a number of animal species including horse and cattle. A large amount of *in vivo* data on the haematotoxicity is available for T-2 but not for DON. Thus, repeated exposure to T-2 toxin induces leukopenia in the cat, mouse, guinea pig, rat, rabbit, monkey and pig. When examined, bone marrow toxicity is also seen. The decreases in red blood cells and platelets are less marked than the effect on the white blood cells. *In vitro* studies have also shown white blood cells to be more sensitive than red blood cells and platelets to the cytotoxic action of T-2 toxin.

There are species differences in the susceptibility to the haematotoxicity of trichothecenes. Thus, T-2 causes coagulopathies due to perturbation of haemostasis in poultry whereas in mammals this is due to thrombocytopenia. *In vitro* studies have shown that erythrocytes from ruminants (cow, sheep, goat, buffalo and deer) but not from monogastric animals (pig, guinea pig, rabbit, rat, mouse and horse), including humans, are resistant to the haemolytic effects of T-2 toxin.

The origins of the haematological disorders observed in *Fusarium* toxin intoxication have been elucidated using *in vitro* tests. Human and murine haematopoietic progenitor cells of the bone marrow (platelets, red and white blood cells progenitor) were found to be very sensitive to the cytotoxicity of trichothecenes. This *in vitro* myelotoxicity was highest for T-2 and HT-2 toxins, whereas DON and nivalenol were much less potent. *In vitro* T-2 can induce apoptosis in haematopoietic progenitor cells but this has not been observed with DON. Although the exact mechanism of trichothecene haematotoxicity is not known, Professor Parent-Massin speculated that an initial destruction of circulating cells was followed by inhibition of haematopoiesis caused by cytotoxicity (possibly apoptosis) to haematopoietic progenitor cells in the bone marrow. Overall, the myelotoxicity was considered highest for T-2 and HT-2 toxins and lowest for DON and nivalenol.

From the discussion it became apparent that the duration of exposure required for bone marrow failure to develop is not known. It is also not known whether the pluripotent stem cells or the haematopoietic progenitor cells are affected *in vivo*, and if so, whether any toxicity is reversible.

Toxicity data relevant to hazard characterisation

Dr. J. Schlatter, Swiss Federal Office of Public Health (CH), gave a summary of the toxicity data relevant to the hazard characterisation of the trichothecenes. In the risk assessment part of the risk analysis, the hazard identification (type of effect) together with the dose-response assessment forms the elements of the hazard characterisation. Together with the exposure assessment this permits the risk characterisation which forms the basis for risk management and risk communication.

Dr. Schlatter mentioned that the EC Scientific Committee on Food (SCF) has evaluated the trichothecenes DON, NIV, T-2 and HT-2 and performed a group evaluation for these compounds (SCF 1999, 2000, 2001, 2002). In addition, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) considered DON, T-2 and HT-2 at its 56th meeting (JECFA 2001a, b). Although several outbreaks of human poisoning related to *Fusarium* contaminated food have been reported, in some cases involving several thousand persons, lack of information on the amount of trichothecenes in the food, lack of consumption data and the potential co-exposure to other mycotoxins precluded the use of these studies by JECFA and the SCF in the hazard characterisation. Therefore the hazard characterisation had to rely on data from animal studies. The no-observed-adverse-effect-levels/lowest-observed-adverse-effect-levels (NOAEL/LOAEL) of the individual trichothecenes and the pivotal animal studies from which they are derived were then presented.

In the rat, the major metabolic route of DON is de-epoxidation to the corresponding methylene derivative. DON and its metabolites are primarily excreted via faeces but occur also in the urine (at 96 hrs 65% and 25%, respectively). The plasma elimination half-life of DON in pigs is about four hours. T-2 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. The plasma half-life for T-2 is less than 20 minutes. The main biotransformation pathway is deacetylation of the C-4 acetyl group of T-2, which leads to the formation of HT-2. There is no toxicokinetic information from humans.

In animals, acute/subacute oral toxicity of DON is characterised by vomiting, feed refusal, weight loss and diarrhoea. Emesis and anorexia are mediated by the serotonergic system in the central nervous system (CNS) or via peripheral actions on serotonin receptors. The minimum emetic oral dose in pigs is in the range of 50 – 200 µg/kg body weight/day. Immunotoxicity, haematotoxicity and necrosis in various tissues such as the gastrointestinal tract, bone marrow and lymphoid tissues are also observed but at higher doses. There are no indications that DON has carcinogenic and/or genotoxic properties. A tolerable daily intake (TDI) of 0-1 µg/kg body weight (or a Provisional Maximum Tolerable Daily Intake (PMTDI) in the terminology of JECFA) was established by the SCF and JECFA based on the NOAEL of 100 µg/kg body weight/day. The effect on body weight (reduced growth) was identified in a two-year chronic dietary study with mice. An uncertainty factor of 100 was applied (SCF 1999, 2002; JECFA 2001a).

The toxicological profiles of NIV, T-2 and HT-2 are similar to that of DON. Also for NIV, there are no indications of carcinogenic properties. However, the database for NIV is much less complete than for DON. A NOAEL could not be identified from the available long-term studies and reproductive studies. The SCF therefore applied a large uncertainty factor of 1000 to a LOAEL of 700 µg/kg body weight/day derived from a 2-year dietary study with mice. Growth retardation and leukopenia were the critical effects seen. This established a temporary TDI (t-TDI) of 0-0.7 µg/kg body weight (SCF 2000). However, since the test material was pulverised *Fusarium nivale* culture material using a strain that reportedly does not produce other trichothecenes, it was not clear whether NIV was really the only mycotoxin tested.

T-2 may be a more potent inhibitor of protein synthesis than DON. Studies in mice and rats indicate that T-2 causes cytotoxicity 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. These effects were observed primarily at concentrations known to inhibit protein- and DNA-synthesis and produce cytotoxicity. T-2 has induced hepatocellular and pulmonary adenomas in male mice.

As T-2 is rapidly metabolised *in vivo* to HT-2 and the acute toxicity of T-2 and HT-2 are within the same range, the toxicity of T-2 might well, at least partly, be attributed to HT-2. Hence, a combined t-TDI for the sum of T-2 and HT-2 was set. Both the SCF and JECFA used haematotoxicity and immunotoxicity of T-2 toxin in pigs in a short-term study of three weeks as the basis for the safety assessment. Slight effects on immune parameters and also a reduction in the feed intake by about 10% were seen at the lowest dose of 30 µg/kg body weight/day. Similar effects were not observed in other studies in pigs neither at this nor at higher doses. This LOAEL was therefore considered to be close to the NOAEL. An uncertainty factor of 500 was used to derive a t-TDI of 0-60 ng/kg body weight (SCF 2001; JECFA 2001b).

Dr. Schlatter emphasised the gaps in knowledge and proposed further studies to fill the gaps. Studies on toxicity, especially neurotoxicity (emesis, anorexia), should be undertaken for the entire trichothecene group to confirm that neurotoxic effects do not occur at doses lower than those affecting growth and body weight. Comparative studies of toxicokinetics and metabolism in different species would be welcomed. A human NOAEL should be determined in epidemiological or clinical studies in order to establish an acute reference dose. For DON a confirmatory carcinogenicity study in the rat was requested. Given the weak database on NIV, genotoxicity studies and a long-term toxicity/carcinogenicity study with doses to derive a NOAEL would be useful. Due to the uncertainty about the carcinogenic potential, properly designed carcinogenicity studies in rats and mice were requested for T-2 and HT-2. These should include pair-fed controls to account for the potential protective effect of reduced feed consumption. The NOAEL should be determined with a 60-90 day study in the most relevant and sensitive species, the pig, using haematological and immunological parameters as the most sensitive endpoints.

During the discussion the question was raised whether DON-induced growth retardation would be most important in young children where exposure is highest. However, the pivotal mouse study started with six weeks old mice (corresponding to about 10 years in humans). It was agreed that this is a general problem in toxicology. The safety factors applied to the NOAEL should account for this uncertainty but the gap can only be avoided if a multigeneration study or a relevant human study is undertaken. The importance of determining acute reference doses for mycotoxins in humans was stressed again. This was considered possible when there is no evidence of carcinogenicity and when the predominant critical effect is acute. For trichothecenes a study defining the emetic NOAEL could be done in primates although a clinical study in humans would be the best solution for strengthening the database and reducing uncertainties.

The metabolism of trichothecenes differs between species. De-epoxidation of DON occurs in ruminants and less predictably in rodents but not in the pig and probably not in humans. Therefore the relevance of rodent data to the human situation was raised. After a lively discussion it was agreed that such differences commonly occur in toxicology. The metabolic fate of toxicants can be influenced by the gut microflora, components in the diet (e.g. antioxidants), or metabolism in other tissues (first passage through the liver markedly detoxifies T-2). Animal data cannot be discarded because of that. The problem is accounted for by selecting the most sensitive endpoint in the most sensitive species and by an inter-species safety factor of 10 being applied to the NOAEL. However, rather than discussing toxicological details such as species differences in metabolism, the uncertainties concerning humans should be reduced (refined assessment of actual exposure, generate more human data).

Combined toxic effects

Dr. G. Speijers, National Institute of Public Health and the Environment (NL), was given the task of evaluating possible combined toxic effects due to the co-occurrence of several mycotoxins in food. It is well known that food items infected by fungi in the field or during harvest and storage can contain concomitantly different mycotoxins (not only trichothecenes). This co-occurrence of mycotoxins can be the result of production by one particular fungal species. It can also result from several fungal species producing different mycotoxins growing at the same time or subsequent to each other. Examples of co-occurrence include citrinin and ochratoxin A, citrinin and patulin, trichothecenes i.e. DON and zearalenone, trichothecenes and fumonisins and fumonisins and moniliformin. The question is whether such a combined intake of mycotoxins could pose a possible higher risk than the intake of one of these mycotoxins alone. Using the potential induction of renal disease as an example, it was demonstrated that a multitude of different factors acting together via similar or different mechanisms could theoretically contribute to disease development. The factors mentioned were mycotoxins (DON, fumonisins, ochratoxin A), infectious agents, endotoxins and oxidative stress.

Combined effects of chemicals in mixtures can be neutral (no effect), additive, synergistic or antagonistic. While a group TDI can potentially cover additive effects within a group of mycotoxins, synergistic effects would require extensive research to support the hazard characterisation and risk assessment. Dr. Speijers stressed that for such interaction studies, the choice of animal species and the dosing schedule (low dose versus high dose) as well as the definition of the pivotal endpoint (the type of interaction may not be the same for all toxic effects) are very important.

The database on combined toxic effects of mycotoxins is generally limited, in particular for trichothecenes. Experimental studies have addressed combinations of ochratoxin A and other mycotoxins but only a few well-designed studies are available. A synergistic effect on kidney cells has been observed for the combination of ochratoxin A and citrinin *in vitro*. *In vivo*, this mixture caused synergistic effects on the kidneys in the miniature swine, dog, female guinea pig, rat and mouse, whereas additive or antagonistic effects were seen in the male guinea pig and poultry.

Very limited experimental data are available for trichothecenes. Taken together, the *in vitro* and *in vivo* data on combined effects of trichothecenes point to additive effects at most, with no synergistic effects. This was to be expected from mycotoxins of similar structure and produced by the same species or families, where the mode of action and the toxicity profiles will be quite similar. In terms of risk assessment, these mycotoxins could be dealt with by establishing a group TDI.

However, during the discussion, the audience was informed that the SCF has attempted to perform a group evaluation of DON, NIV, T-2 and HT-2 (SCF 2002). The SCF found that dose additivity, as well as antagonism has been observed for T-2 toxin, DON and nivalenol *in vitro*. *In vivo* only antagonism was observed for T-2 toxin and DON and no dose additivity was observed. Although there were indications for dose additivity in some of the *in vitro* studies, the database was not sufficient for the SCF to establish the relative potencies of the trichothecenes in support of a group TDI for these trichothecenes.

Several attendees stressed that studies on combined effects of multiple chemical compounds, such as pesticides and industrial chemicals, have shown that the dose administered is very important. Synergism may prevail at high doses and additive or neutral effects at low doses. Results of animal toxicity studies have not demonstrated any toxicity following combined exposure to arbitrarily chosen chemicals when all chemicals in the mixture were administered at dose levels slightly lower than their own individual NOAEL. This was valid also for combinations of

chemicals that have either different target organs and/or different target sites within the same organ (i.e. differ in the mode of action). Therefore, exposure to such mixtures is not associated with a greater hazard than exposure to the individual chemicals, provided that the exposure levels are at or below the individual NOAELs. Certainly, when the exposure levels are at the ADI/TDI levels, no greater hazard is to be expected. At exposure levels higher than the NOAEL, both synergistic and antagonistic effects may be seen, depending on the compounds. The use of the term addition is only scientifically justifiable when all the chemicals in the mixture act in the same way, and by the same mechanism, and thus differ only in their potencies.

In conclusion, it was agreed that synergistic interactions between trichothecenes are not to be expected at the generally low levels of human exposure and that research in this field should not have priority. The real threats concerning additive/synergistic effects may be combinations of exposure to mycotoxins and to infectious agents (e.g. aflatoxins and hepatitis B virus).

SESSION 4: SAMPLING AND ANALYTICAL METHODS

Rapporteur: Dr. J. Hunt, Kraft Foods (D).

Sampling for trichothecenes analysis

Dr. J. Stroka, Joint Research Centre (B), opened his presentation on sampling methods for the analysis of DON by reminding the audience that a correct analytical result cannot be obtained without representative sampling. Furthermore, the sampling must be feasible for it to be widely implemented. This is especially a challenge for mycotoxins, which tend to be distributed very heterogeneously throughout the sample in so-called “hot-spots”. Much effort has been made to establish statistically and mathematically correct sampling plans. To date, this work has focused on aflatoxins, since legislative limits have existed in many countries for several decades. This is reflected in European Directive EC/53/1998 (amended by Directive EC/27/2002) concerning methods of analysis and sampling for the official control of aflatoxins. This sampling plan results in a maximum aggregate sample of 30 kg (100 samples of 300 g), depending on lot size. Ochratoxin A is less heterogeneously distributed than aflatoxins and the maximum aggregate sample as defined in Directive EC/26/2002 is 10 kg. DON is also considered to be less heterogeneously distributed compared to aflatoxins.

In Europe 200 million tons of grain are traded through many ports of entry per year. With the current official sampling plans only 4%–25% thereof can be monitored owing to issues of logistics, sample treatment such as milling and preparation of sub samples, loss of material and sample disposal. To address this issue, Dr. Stroka explained how the Joint Research Centre (JRC) and Institute for Reference Materials and Measurements (IRMM) are exploring simple and rapid screening approaches, which combine effective sampling with analysis for bulk commodities. Building on the premise that fungal growth occurs on the surface of the kernel and that mycotoxin contamination is present on the outside of the kernel, the same technologies as those used to screen aircraft luggage for explosives have been explored to detect mycotoxins. Essentially the surface material is agitated (e.g. by vacuum) to create a dust, which is representative of the surface material. This dust can then be analysed using various rapid techniques. Results for ochratoxin A in green coffee, aflatoxins in pistachio and DON in maize were presented.

During the discussion, it was agreed that such investigations are highly valuable to deliver a rapid result provided representative sampling was ensured. They could highlight highly contaminated lots, which would then undergo further analyses. However, it was also agreed that further data are required to understand the quality of the correlation between the mycotoxin levels in dust and actual levels in the sample.

State of the art of trichothecenes analysis

Dr. P. Koch, Swiss Quality Testing Services (CH), opened her presentation on the state of the art of trichothecenes analysis by explaining that there is no routine method available to simultaneously detect the type-A trichothecenes, which include T-2 toxin and HT-2 toxin, and the less polar type-B trichothecenes, which include DON, NIV, 3-acetyl DON and Fusarenone X, with a sufficiently low limit of detection. However, LC-MS methods are being developed and there are already studies published that report simultaneous detection of A- and B-trichothecenes. The difference in polarity between A- and B-trichothecenes should not be overemphasized since this is not the major reason for the use of different analytical methods. The reasons are lack of the conjugated carbonyl-double-bond system and the lack of UV-absorptivity as a consequence of different functional groups.

Thin layer chromatography was the first analytical methodology to be applied to the analysis of trichothecenes. Techniques used more recently include gas chromatography (GC) (with electron capture, mass spectroscopy or flame ionization detection), liquid chromatography (LC) (with fluorescence or mass spectroscopy detection) and enzyme linked immunosorbent assay (ELISA). Molecular imprinted polymers (MIP) are being investigated to deliver a high level of DON enrichment for clean up.

In reviewing the published literature, Dr. Koch remarked that 90% of the published data for Type-A trichothecenes use GC methodology with the lowest limits of detection reported at 2 µg/kg. Similarly for Type-B trichothecenes, GC methods are mostly used (75%) with limits of detection reported at 10 µg/kg. There is now increasing use of LC technologies using mass spectroscopy (MS) detection systems, especially for the Type-B trichothecenes. Limits of detection tend to be slightly higher than for GC methods.

Whether GC or LC methods are used, appropriate sample preparation (clean-up) is required. In the case of complex matrixes such as foods, immuno affinity columns (IAC) are most effective. Dr. Koch presented results for DON from various studies using both GC and LC methods. Whilst having low limits of detection, GC-methods have been shown to suffer from poor comparability of measurement results for DON. An LC method with no derivatisation and IAC clean up could simultaneously detect DON, 3-acetyl DON and 15-acetyl DON, however with higher limits of detection than generally found with GC methods. The UK Food Standards Agency has recently run a survey for DON in various flours (oat, rice, wheat), polenta and breakfast cereals where an LC method with UV detection (220 nm) was employed. Excellent intra- and inter-laboratory deviations were reported.

Dr. Koch concluded that currently there are a number of methods that can be applied to the analysis of DON and other trichothecenes. The choice of method to be applied is dependent on the nature of the test, i.e. it must be fit-for-purpose. For example, if analysis is to be performed to check against legal limits, the limit of detection/quantification must be sufficient.

During the discussion, the importance of fit-for-purpose methods was further emphasised as well as the need to report the recovery and the corrected value in order to better compare results between laboratories. To further improve comparisons between laboratories there is a need for the development of common internal standards. Regarding IAC, it was noted that columns should be checked in the laboratory by performing recovery experiments. This is necessary since it is not uncommon that during transportation the columns are subjected to unsuitable temperature fluctuations, which affect performance. It was further noted that MIP columns are still at the very early stages of development and are not yet commercially available.

Rapid methods

Professor E. Usleber, University of Giessen (D), opened his presentation on rapid methods for the detection of DON and other mycotoxins by outlining what is expected of rapid methods. The criteria include: portability, limited instrumentation, low technical skill requirement, simple and reliable robust yes/no answers and low cost. Professor Usleber also highlighted that the rapidity of a method was a function of the number of samples analysed in one batch. For one-off samples, dipstick and microtiter plate technologies are much more rapid than LC methods. However, this difference decreases as the number of samples to be analysed increases. The same is true for the relative workload and cost advantages of the rapid methods compared to the more sophisticated chromatographic methods.

As TDIs are established and legislative limits set for various mycotoxins, it is evident that rapid methods must be able to operate at high sensitivities. Antibody-based techniques lend themselves to operating under the criteria of rapid methods as well as the low limits of detection. However, appropriate antibodies are not available for all the mycotoxins for which a TDI exists, e.g. not for NIV. Processed food presents a significant challenge for antibody-based methods. They are more suitable for pre- and post-harvest analysis, at which time rapid/robust testing is required as a means to decide on the future use of a cereal lot. In the laboratory, microtiter plate ELISA still offers significant benefits in terms of speed, sensitivity and quantification. Professor Usleber's laboratory has evaluated such techniques for detection of DON in infant foods, bread and noodles with limits of detection from 15-30 µg/kg, and beer with a limit of detection of 2 µg/kg.

In non-laboratory environments, e.g. in the field or at truck unloading stations, dipstick and lateral flow technologies that give a rapid visual result are more appropriate. However, the price of simplification and acceleration demanded by these techniques is a loss of sensitivity. Professor Usleber presented results of lateral flow dipstick enzyme immunoassays for DON. Positive results were found for spiked DON levels higher than 0.5–1.0 mg/kg. Naturally contaminated wheat and noodle samples gave positive results at DON levels of 1.2 and 3.2 mg/kg, respectively. In conclusion, Professor Usleber commented that these dipsticks are not appropriate for end product control of food but may be appropriate for pre- and post-harvest control. To further improve performance characteristics of rapid analytical tools, better antibodies and signal transduction are required. Additionally, sampling and preparation techniques need further improvement. Looking to the future, Professor Usleber mentioned work on developing multi-mycotoxin ELISA tests and also biosensors, although work here is very preliminary.

During the discussion, the use of near-infrared-spectroscopy (NIR) as a rapid method was proposed. It was mentioned that good correlations had been found for DON in wheat, and that it could be a promising method for identifying highly contaminated lots. Barley tended to have higher limits of detection and more variance compared to wheat samples. The advantage of NIR is that no sample preparation is required, however, it is an empirical method requiring careful calibration and validation.

It was also suggested that since the dipstick technologies analyse a crude extract, the use of a clean-up method could help to improve results, as long as the method remained fit for purpose considering the balance between appropriate speed and quality of results.

Reference materials and validated methods

As maximum tolerable levels for mycotoxins are being established, it is important that methods to check compliance against these are appropriately validated. Certified reference materials (CRM) play a key role in the validation. Dr. R. Josephs, Joint Research Centre (B), explained how the IRMM (Institute for Reference Materials and Measurements) is committed to method validation and the production of (certified) reference materials aligned to the implementation and monitoring of national and European legislation and standardisation. This work delivers an essential tool for comparability and traceability, fulfilling the requirements of ISO/IEC 17025 and enabling trueness of methods to be established (which is possible only with a certified reference material). Once a method is validated, its performance characteristics are defined (e.g. precision, linearity, limit of detection and quantification), enabling a judgment to be given regarding its suitability. This is critical since, whereas in the past a standard method would be prescribed, performance characteristics are now more generally defined. For example, the European Committee for Standardisation (CEN) Technical Committee – Biotoxins has established minimum requirements for the performance characteristics that mycotoxin analysis methods should meet, depending on the level of contamination. For DON and NIV, analytical methods intended to be used for concentrations of >100 µg/kg are required to have a recovery in the range of 100% and relative between laboratory standard deviation (RSD_R) values of <20% and <40% respectively (European Committee for Standardisation, 1999).

After explaining the critical steps in the development of CRM, from demand to post-certification monitoring, Dr. Josephs went on to give an overview of previous method validation activities for DON. He concluded that generally there was poor agreement of results as evidenced by high CV% values. Agreement was somewhat improved when common calibrants were used compared to individual laboratory calibrants. In response to this, a feasibility study is underway for the production of certified calibrants for the determination of DON and other type B trichothecenes (DON calibrant G6RD-CT-2002-853). Additionally, a European working group "Method of Analysis–Deoxynivalenol," has been established to perform an inventory of analytical methods for DON, review available validation data, select appropriate methods and organise interlaboratory studies to deliver a validated method for DON analysis. In addition to the projects outlined above, other IRMM activities involve producing new CRM for DON, NIV, T-2 and HT-2 toxins. This includes wheat and maize materials at very low (< 20 µg/kg) and medium levels (200-500 µg/kg), as well as calibrant solutions (about 20 µg/ml).

During the discussion the importance of validated methods and the development of calibrant CRM was re-emphasised. The ongoing project work to address these topics was applauded as addressing the current gaps. Further points raised included the need for specifying the uncertainty of the measurement and indicating the recovery for each delivered result. Whilst this is a requirement under ISO 17025, this is not always practiced and can lead to erroneous interpretation of the data.

When analysing data for exposure studies, it is common practice to report "non-detectable" results as 50% of the limit of detection (LOD). This becomes an issue especially when the limit of detection is close to the TDI, leading to erroneous exposure estimates. This reflects the requirement that a method must be fit-for-purpose. In the case of exposure studies, methods with very low limits of detection are desirable. Other approaches to manage "non-detectable" results were also discussed, such as to assume a certain distribution of values below the limit of detection. In this case the nature of the distribution needs to be understood. Alternatively, "non-detectables" could be treated both as zero and as the limit of detection, thereby giving a range of exposures from best to worst-case scenarios. Approaches actually applied today are variable, and it was concluded that a common harmonised approach to handling "non-detectable" values needs to be agreed on when assessing exposure.

In conclusion, the group recognised three areas in which methods must be validated and proven fit for purpose. These included (i) instances where data are to be used for compliance checks, where the limit of detection/quantification needs to be appropriate to the legislated limits; (ii) instances where data are to be used for exposure studies, where the limit of detection should be as low as possible (especially in relation to the TDI); and (iii) instances where data are to be used for rapid screening, where the risk to sellers/buyers needs to be transparent and agreed.

SESSION 5: ASSESSMENT OF EXPOSURE

Rapporteur: Dr. J. Hunt, Kraft Foods (D).

Critical assessment of exposure – report from SCOOP project

In 2001, the 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” (Gareis *et al.* 2001), was established. The task was divided into three subtasks: zearalenone, fumonisins and trichothecenes. In his talk on critical assessment of exposure Dr. R. Schothorst, National Institute of Public Health and the Environment (NL), presented the results from the trichothecenes subtask that was co-ordinated by The Netherlands.

Overall, almost 35,000 results were received covering the occurrence of 12 different trichothecenes in 12 different countries. However, most of the data covered DON, NIV, T-2 and HT-2 toxins. Since these are the trichothecenes for which the SCF had set TDIs, only data for these mycotoxins were discussed. Data were obtained from laboratories with a quality system in place. Most data had been collected for wheat samples, but among the cereals, maize showed the highest level of contamination with the trichothecenes.

Most occurrence data had been collected for DON (over 11,000 results) and in 57% of the samples, positive results were reported. With reference to the proposed EU guidance limits, 6% of products were > 500 µg/kg and 7% of cereals and flours were > 750 µg/kg.

Over 4,000 samples had been analysed for NIV and positives were found in 16% of the cases, with a consistently low occurrence amongst the different cereals. For T-2 over 3,400 samples had been analysed, with 20% showing a positive result. However, Italy reported positive results for over 90% of the samples. Over 3,000 samples were analysed for HT-2, with 14% showing positive results and in this case oats had the highest contamination occurrence.

Eleven countries delivered food consumption data. Methodology varied greatly between the different countries and included 1- and 7-day dietary surveys (recall and diary), food balance sheets and individual household data. Dr. Schothorst commented that there is a significant lack of consumption data in some countries. In particular he noted that information on babies' and children's food intake is generally not available.

Intake data were delivered by 12 countries and presented as mean intake (mean consumption x mean occurrence) and high intake (95% level). Non-detectable data points were taken as 50% of the limit of detection. Wheat and wheat-containing products such as bread and pasta represented the major source of intake for the four trichothecenes. The estimated mean intake for DON was below the TDI, however, for young children they were close to the TDI. When comparing the high intake levels with the TDI, it is clear that most of the intakes are above the TDI, especially those for young children. For NIV, mean and high level intakes for all groups were well below the t-TDI. Summarising the data for HT-2 and T-2 toxins together, in most cases the estimated intakes (mean as well as high intakes) were above the t-TDI. Dr. Schothorst commented that since only a low level of positive results was reported for T-2 and HT-2 (20% and 14%, respectively), the majority of data points for occurrence were input as 50% of the limit of detection of the analytical method. This could lead to an overestimation of the total intake.

As areas for future work Dr. Schothorst identified the need to develop methods with lower limits of detection, consumption data for specific population groups (especially children) and a common approach to assess dietary intake.

During the discussion, the need to have consistent reporting of results was again highlighted, i.e. data collected for recovery, and uncertainties should be reported. Building on discussions in the previous sessions, the issue of how to report non-detectable data was emphasised. The data for T-2 toxin clearly demonstrated this point. In this case 80% of the data points were non-detectable, and application the 50% LOD rule leads to an unreliable estimate of the intake.

The consensus was that there are good data on DON because of the large number of samples. For NIV, there is currently no issue since intake is well below the t-TDI. However, for the other mycotoxins, there is not enough data to give reliable intake data and more work is needed, especially on T-2 toxin, including the harmonisation of how to handle non-detectable results.

Probabilistic exposure models

In her talk on risk assessment and risk management of DON in The Netherlands, Dr. M. Pieters, National Institute of Public Health and the Environment (NL), presented the use of probabilistic exposure and effect assessment to assess DON intake and possible health effects for two time periods in the Netherlands. The first period was from September 1998 to January 2000, and reflects high contamination. Levels up to 2,000 µg/kg of DON were detected in wheat and wheat products in 1998 and 2000. The second period was February 2000 through December 2002. As a result of increased monitoring and introduction of risk management measures (e.g. action limits to exclude highly contaminated lots from being used in the production of human foods) the high levels previously found were not seen in the second period.

The mean DON concentration in wheat for the 2000-2002 period was approximately 50% of the mean concentration in 1998-2000. This was also reflected in the lower concentration for a range of food products and particularly for bread and baby food. The measured or estimated concentrations of DON in the food products were combined with the individual consumption data obtained from the Dutch National Food Consumption Survey. To distinguish the variation between individuals from the daily fluctuations in consumption, a statistical exposure model (STEM) was used. As a general result, it was found that the relative intake decreases with age, with the one-year old child having the highest estimated intake of DON. As a result, it was estimated that the intake of DON for the period 2000-2002 was one-third that for the period 1998-2000. During 1998-2000, 80% of one-year old children were estimated to exceed the TDI of 1 µg/kg bw for DON. For the latter period, this was reduced to 5% and the estimated mean intake for one-year old children was 0.45 µg/kg bw/day (95th percentile: 1.00 µg/kg bw/day). At the age of 10 years (and higher), the TDI was no longer exceeded.

The data from a 2-year feeding study with mice were used for the probabilistic effect assessment. In this study, the pivotal effect of DON was reduced body weight gains in male and female mice. The dose-effect relationship in male and female mice could be described by one single regression function, with a different parameter value for each sex. Monte Carlo sampling was used to generate a large number of data sets from this regression model, each time with the same number of data points per dose group as the number of animals in the experiment. For each of these data sets a critical effect dose (CED) was estimated. Extrapolation to humans was carried out by combining the distribution of the CED with distributions of the assessment factors for inter- and intra-species variation in a probabilistic manner. Taking the exposure in the human population, an expected effect in sensitive humans was developed from which the possible risk was derived.

Thus, at a DON intake of 1 µg/kg bw/day (the 95% percentile intake of DON for a one-year old child), a 1% change in body weight might be the best estimate. It was estimated that a reduction in body weight would be unlikely to exceed 9%.

Overall, in considering that a 10% reduction of body weight was borderline between adverse and non-adverse and noting that the exposure to DON declined rapidly with increasing age, Dr. Pieters concluded that, based on her analysis, the current exposure levels to DON do not seem to give rise to clearly adverse health effects in children.

During the discussion, it was remarked that combining dose-response modelling with probabilistic approaches delivers a quantitative risk assessment. This has an advantage for risk managers especially where no threshold can be applied (as is the case for genotoxic substances). Furthermore, it allows for the uncertainty of the data to be included thereby giving an extra level of confidence.

There was discussion about the amount and range of data required for the dose-response curves. It was remarked that tighter dose spacing with more dose groups would allow for a better estimate of the benchmark dose.

Also recognised as areas for improvement were studies to better understand inter- and intra-species differences with respect to dose-response, especially the difference between adults and children. In this context it was commented that it should be possible to obtain data on adverse effects to drugs which could allow for developing more precise data on intra-species and possibly adult/child differences.

SESSION 6: RISK ASSESSMENT AND PREVENTION STRATEGIES

Rapporteur: Dr. J.C. Larsen, Institute of Food Safety and Nutrition (DK).

Risk assessment paradigm and its application for trichothecenes

In his talk about the risk assessment paradigm and its application for trichothecenes Dr. S. Page, World Health Organisation (CH), explained that risk assessment is constrained by uncertainties associated with the lack of adequate data, and risk management must consider the fact that contamination can have serious impacts on trade and food sufficiency. These factors necessitate good communication between the risk assessors and risk managers in formulating the questions to be addressed by the risk assessment. He also stressed that the risk assessment must be an iterative process composed of four steps: hazard identification, hazard characterisation, exposure assessment and risk characterisation. Dr. Page then went on to apply this paradigm to the trichothecenes, making reference to the evaluations previously carried out by JECFA (Joint FAO/WHO Expert Committee on Food Additives) and the SCF (Scientific Committee on Food).

Hazards associated with trichothecenes were first recognised from livestock toxicoses caused by contaminated feed. As studies in experimental animals and *in vitro* were carried out, other adverse effects were identified. The adverse effects thus far identified are emesis, anorexia, immuno-stimulation and -suppression, haematotoxicity, cardiovascular toxicity, gastric, bone marrow and lymphoid necrosis, teratogenesis, reproductive toxicity, neurotoxicity and compromised resistance to infection. The proposed mechanism of toxicity is inhibition of protein synthesis and induction of apoptosis.

The hazard characterisation includes dose-response considerations and evaluation of the relevance of the endpoints observed in experimental systems for humans and no-observed-adverse-effect-levels (NOAEL) or lowest-observed-adverse-effect-levels (LOAEL) for the pivotal effects is determined. At this stage a tolerable daily intake (TDI) may be derived. The pivotal effects for DON, NIV, T-2 toxin and HT-2 toxin are growth retardation, haematotoxicity and effects on the immune system. Since there is currently no convincing evidence that any of the trichothecenes are genotoxic, a threshold for toxicity is generally accepted. Based on the overall quality of the database, a safety factor that takes into account species differences and variations in humans is selected to establish the threshold for the adverse effect in humans.

The evaluations by JECFA and the SCF may be summarised as follows:

Compound	Critical Effect	LOAEL/NOAEL (mg/kg bw/day)	Uncertainty/safety factor	TDI (PMTDI or t-TDI) µg/kg bw/day
DON	Growth retardation	0.1 (NOAEL)	100	1
NIV	Growth retardation Haematotoxicity	0.7 (LOAEL)	1000	0.7
T-2 Toxin and HT-2 Toxin	Immunotoxicity Haematotoxicity	0.03 (LOAEL)	500	0.06

Dr. Page recommended the following additional studies in order to reduce uncertainties in the risk characterisation:

- DON: additional genotoxicity studies and a carcinogenicity study in a second species.
- T-2/HT-2: additional carcinogenicity study and a 60-90 day study (in pigs) for NOAEL.
- NIV: additional genotoxicity studies and a long-term study for NOAEL.
- All: Neurotoxicity studies; comparative studies of toxicity and toxicokinetics in several species; studies on combined effects; long-term low dose studies on immunotoxicity/haematotoxicity.

Because the available epidemiological studies lack definitive dose-response information, in particular that related to acute toxicity, more detailed analytical epidemiological studies of human disease are needed in those areas of the world where the presence of scabby wheat or mouldy maize is a cyclic, endemic event. This would allow the establishment of a dose-response relationship between the intake and acute illness, and enable identification of a NOAEL based on human data.

The quantitative evaluation of the likely intake of trichothecenes on an international basis is extremely problematic. Food consumption patterns vary tremendously. Also, production and processing practices vary. In addition, data for exposure assessment for subpopulations such as children, pregnant women or the elderly are not available. Most mycotoxin risk assessments by JECFA have been for chronic exposure. In the case of DON acute exposure assessment is also important due to the acute toxicity. Historically, JECFA has had mainly pooled data on mycotoxins available. Intake estimations for the trichothecenes were thus based on a combination of mean food consumption levels with weighted-mean contamination levels of the five GEMS/Food (Global Environment Monitoring System – Food contaminants Monitoring and Assessment Programme) regional diets (African, European, Far Eastern, Latin American and Middle Eastern).

When the TDIs were compared with the estimated exposures in the risk characterisation there were indications that for DON the TDI may have been exceeded in certain regions of the world or for certain population groups. However, for exposure above the TDI health risks depend on the duration and magnitude of excess intake, the nature of the critical effect and slope of the dose-response curve relative to the toxicological database used to derive the TDI, reversibility of the critical effect, toxicokinetics and toxicodynamics.

These risk characterisations include considerable uncertainties. There are inadequacies in intake data, lack of information on toxicokinetics and mechanisms of actions and lack of comparative potency data. For DON there are indications of acute toxicity at relatively low levels. The establishment of an acute reference dose (ARfD) needs to be considered. Moreover, since the trichothecenes are present as mixtures and exhibit similar toxicity, the establishment of group TDIs and toxic equivalency factors should be addressed.

Dr. Page ended his talk by summarising what is known, what is suspected and what is not known. It is known that DON is a frequent contaminant of maize and small grains, that T-2 toxin is a frequent contaminant of small grains in Europe and that the trichothecenes cause a variety of toxic effects in animals and *in vitro*. It is also known that the mean dietary intake of DON and T-2/HT-2 toxins are usually less than the TDIs for the population as a whole in Europe and North America. However, some subpopulations like infants and children may exceed the TDI with some regularity. It is suspected that DON has caused outbreaks of gastrointestinal illness in humans; that T-2 toxin was involved in several (historical) outbreaks of severe human toxicoses; that for DON there are some indications of acute toxicity at relatively low levels, and that at low levels of intake, most of

the effects appear to be reversible. The effects of chronic low dose exposure are still unknown as accurate intake information (especially outside Western Europe and North America); information of exposure to multiple factors (including toxic equivalency factor considerations); information on relevance of acute exposure in humans; data on dose response relationships; information on the significance of excursions above the TDI and more information on factors that contribute to increased susceptibility such as nutritional deficiencies, still needs to be collected.

During the discussion, the need for setting an acute reference dose for DON was again emphasised. However, it became apparent that there is no ongoing research in this area. It was mentioned that ecological human data should not be completely disregarded. It is useful information and DON is a major constituent. Another neglected area is the potential toxicity of trichothecenes by inhalation, which may be higher than after ingestion. This is especially relevant for workers handling contaminated grain and significant research is being undertaken in Canada and Finland.

Prevention strategies

Professor N. Magan, University of Cranfield (UK), talked about prevention strategies for trichothecenes. In the last five years concerted efforts have been made to examine the potential for effective control of trichothecenes, especially DON, from entering the human and animal food chains. *Fusarium* species are responsible for the serious disease called *Fusarium* head blight (FHB), which can result in significant losses in crop yield and quality. FHB resistant cultivars have not yet been developed. Measures that are taken against *Fusarium* development in the field are mainly to control FHB rather than to prevent the mycotoxin risk. Many fungicides are ineffective against *Fusarium* head blight and, more importantly, against toxin accumulation. Recent studies have been carried out in the framework of Hazard Analysis Critical Control Point (HACCP) approaches to identify critical control points (CCPs) in the food production process. In Europe, the main foods of interest are wheat, barley and oats (contaminated by *Fusarium culmorum* and *F. graminearum*) and maize (contaminated by *Fusarium graminearum*, *F. verticillioides* and *F. proliferatum*). Information on the key components in wheat production was addressed and included both pre-harvest and post-harvest factors.

Important pre-harvest factors are choice of variety, GAP (Good Agronomic Practices), weather conditions during flowering, type and amount of fungicides used and moisture content at harvest. Important post-harvest factors are efficient grain drying and sufficient storage capacity, hygiene at storage and management of the stored grain in the short to medium term. The development of resistant cultivars is an important area since it has been shown that wheat cultivars with resistance to the most aggressive, high DON-producing strains of *Fusarium graminearum* and *F. culmorum* inhibited both disease progression and toxin production.

Appropriate agronomic practices include deep ploughing as a method to remove residual fungal material from the surface and crop rotation, intended to break the production of infectious material. However, the use of maize in rotation should be avoided. The weather conditions, especially moisture and temperature, at anthesis (flowering) are critical in *Fusarium* infection of the grain. Professor Magan stated that moisture and temperature relationships and limits for germination, growth and DON/NIV production are now known for wheat. Recent work has shown that agrometeorological information preceding and during ripening can be used for predicting risk of DON contamination of wheat by *Fusarium graminearum* and *F. culmorum*. This could provide the basis for decision support systems for predicting risk and hence taking preventive action, such as effective timing of fungicide use. Regarding fungicide use, studies have found that full dose application of fungicides with main activity against *Fusarium* species

timed at mid-anthesis were most effective at inhibiting *Fusarium* head blight, DON and NIV production. Professor Magan also mentioned studies indicating that certain biocompetitive microorganisms may prevent *Fusarium* infection of cereals and maize when applied to flowering ears.

Harvest is the first stage in the production chain where moisture management becomes the dominant control measure in the prevention of mycotoxin development. Another important control measure at this stage will be an effective assessment of the crop for the presence of disease accompanied by an efficient strategy for separation of diseased material from healthy grain. Generally, grain stored at moisture contents equivalent to less than $0.70a_w$ (< 14.5% moisture by weight) will not be subject to fungal spoilage and mycotoxin production. However, grain is often harvested at moisture levels far in excess of this and is often traded on a wet weight basis. Regular and accurate moisture determination during storage as well as efficient and prompt drying of wet grain therefore becomes essential.

Finally, Prof. Magan stressed that prevention is better than cure. The most important tools in the prevention would be decision support systems based on weather forecasting at anthesis, rapid diagnostics for detection of toxins in the food chain and effective post-harvest drying of grain.

In the discussion, Prof. Magan mentioned that the ISPRA Monitoring Agriculture with Remote Sensing (MARS) Unit in corporation with JRC and the International Programme on Chemical Safety (IPCS) issues EU-MARS reports on weather/crop conditions in Europe (<http://mars.aris.sai.jrc.it>). As regards GAP, the audience was made aware of the Codex draft code of practice for the prevention of mycotoxin contamination in cereals, including annexes on ochratoxin A, zearalenone, fumonisins and trichothecenes (Alinorm 03/12A 108 Appendix).

SESSION 7: FINAL DISCUSSIONS AND CONCLUSIONS

Rapporteur: Dr. J.C. Larsen, Institute of Food Safety and Nutrition (DK).

The workshop brought together experts from relevant areas of mycotoxin research and risk and exposure assessment. The discussions reflected the mixture of disciplines represented. In the following section, overall discussion points and conclusions are described under five different headings: prevention, sampling and analytical methods, exposure assessment, toxicology and exposures above the TDI. It has to be stressed that the time available at the workshop did not allow for any priority setting.

Prevention

It was agreed that the strategies for the prevention of *Fusarium* infections of grains should be based on the principles of HACCP from the field to consumption. The post-harvest stages in the commodity chain, which include drying, storage, transport, milling and food processing, are more conducive to the classical type of HACCP analysis than the pre-harvest stages. This is because they are characterised by the ability to apply definitive control measures, to set critical limits and to initiate monitoring. The following points were considered:

Selection of cultivars:

The increased use of high-yielding wheat varieties with a greater susceptibility to *Fusarium* infection has increased the *Fusarium* problem in Europe. The selection of cultivars should be based on mycotoxin resistance as well as high yields. At the same time, resistant varieties should be evaluated to ensure that they do not themselves cause problems by producing toxic secondary metabolites.

Agricultural practices:

Codex has prepared a draft code of practice for the prevention of mycotoxin contamination in cereals. However, it is still important to better understand which agronomic factors affect mycotoxin contamination of grain so that good agricultural practice can be continuously improved in order to minimise contamination. Deep ploughing to remove residual fungal material from the soil surface and crop rotation to break the production of infectious material are preventive factors. The use of maize in rotation should be avoided. Moisture at anthesis favours *Fusarium* infection of the grain, and the most critical period pre-harvest is a narrow window bridging the flowering and ripening of the grain. Decision support systems, based on weather forecasting in the periods preceding and during ripening, were advocated and recommended for predicting risk. This would allow growers to take preventive action, such as effective timing of fungicide application or of biological control agents.

An important control measure at harvest is the assessment of the crop for the presence of disease, accompanied by an efficient strategy for separation of diseased material from healthy grain.

Moisture control:

Moisture management at harvest and during storage and transport is the most important control measure in the prevention of mycotoxin development. Generally, grain stored at a moisture content less than $0.70a_w$ (< 14.5% moisture by weight) will not be subject to fungal spoilage and mycotoxin production. Regular and accurate moisture determination during storage, as well as efficient and prompt drying of wet grain is essential. This requires the availability of sufficient and proper storage buffer and drying facilities at grain collection points.

Currently, grain is traded on a wet weight basis – a practice that will have to be reviewed as it does not encourage producers to dry the grain.

Processing:

Wet milling of cereal grains in most cases results in reduced trichothecenes content in the fractions used for food production. In the case of dry milling of maize the highest levels of toxins occur in germ and bran fractions with less in white flour and grits – the streams that enter the food chain as breakfast cereals, snacks and polenta. After dry milling of wheat, the highest concentrations of trichothecenes are found in the bran and wheat feed with lowered concentrations in white flour.

Trichothecenes are relatively heat-stable chemicals with high water solubility and surveillance of retail foods of cereal origin demonstrates that trichothecenes do survive production processes, such as baking and extrusion. However, during cooking of pasta, DON was lost to the cooking water.

Sampling and analytical methods

It is important that representative samples are selected and analysed thereby delivering a representative result. Owing to the heterogeneity of mycotoxin distribution within samples validated sampling protocols tend to involve large quantities of sample (e.g. up to 30 kg for aflatoxins). As this impacts the actual amount of testing that takes place, it is recommended to develop more feasible sampling plans. Building on the premise that mycotoxin contamination is largely on the surface, investigations are underway to determine the efficacy of agitating the surface material and analysing the resulting dust by rapid methods. It was recognised that such combined sampling and analysis techniques could deliver an initial screening of bulk commodities to identify highly contaminated lots, which would then be further analysed.

There is no single analytical method that is applicable to all of the trichothecenes – as a result of their considerable range in polarity. The method used should be fit-for-purpose. Analytical data are used for three purposes and this defines which is the most appropriate method to use.

Screening:

This may involve testing at truck unloading points where reliable screening methods of low cost are required. Dipstick and lateral flow kits are useful for delivering yes/no answers. However, sensitivity is compromised and extremely good antibodies are required. For rapid testing in the laboratory, ELISA microtiter plates deliver fast and quantitative results.

Control:

Valid methods are being developed for regulatory purposes. In this case it is essential that the limit of detection and limit of quantification of the method be below the maximum allowed value. In addition, rapid generation of results is desirable to enable samples to be released. The number of samples generally analysed can also drive the appropriate method. For high sample throughput, the initial high costs of GC or LC equipment may be offset over time, whereas ELISA microtiter plates may be a more appropriate solution for low sample volumes.

Exposure:

Owing to the problem of how to handle non-detectable results when performing exposure studies, it is important that the methods used are those that deliver the minimum limit of detection/quantification. In this case GC and LC methods are most suitable. GC methods tend to deliver lower limits of detection compared to LC methods. It was recognized that there is an urgent need to agree on how to handle non-detectable data for the purpose of exposure studies. The most used practice is to report non-detectable results as 50% of the limit of detection. However, there was general consensus that this is not a scientifically valid approach. Another option discussed was to model the distribution of mycotoxin contamination below the limit of detection thereby requiring knowledge of the nature of the distribution.

As a general point to all methods, sampling must be defined and methods validated against performance requirements so that where accept/reject decisions are made there is transparency for all parties concerned. Furthermore, certificates of analysis must comply with ISO 17025 and indicate the uncertainty and the value corrected for recovery.

As a means to enabling appropriate method validation, including determination of trueness, certified reference materials (CRMs) are available for DON in maize and wheat flour. However, it will be necessary to produce CRMs for DON, NIV, T-2 and HT-2 toxins in maize and wheat flour at very low (< 20 µg/kg) and medium (200 – 500 µg/kg) concentrations.

Exposure assessment

The 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” provided mean (mean consumption x mean occurrence) and high (95% level) intake data for trichothecenes from 12 member countries. The estimated mean intakes for DON were generally below the TDI. When comparing high intake levels with the TDI, it appeared that for young children most intakes were above the TDI. Mean and high level intakes of NIV for all groups were well below the t-TDI. Summarising the data for HT-2 and T-2 toxins together, in most cases the estimated intake (mean as well as high intakes) were above the t-TDI. The majority of data points for occurrence were input as 50% of the limit of detection of the analytical method. This could lead to an overestimation of the total intake.

In a recent investigation from the Netherlands using probabilistic exposure and effect assessment, a significant decrease in DON occurrence in foods and hence in the intake of DON was reported for the period 2000-2002 as compared to 1998-2000. This resulted from increased monitoring and introducing risk management measures. The intake of DON for the period 2000-2002 was one-third that for the period 1998-2000. For the period 2000-2002, 5% of one-year old children were estimated to exceed the TDI of 1 µg/kg bw for DON. The estimated mean intake for one-year old children was 0.45 µg/kg bw/day (95th percentile: 1.00 µg/kg bw/day).

The exposure assessment integrates occurrence data with consumption data. In both areas there is a clear need for collection of better data in order to provide realistic exposure estimates and comparisons within and between countries. It was therefore recommended that data collection be harmonised. For compounds like DON, collection of acute intake data is also important. While the analytical chemical methods are expected to become more and more reliable in the future and to integrate as many toxins as possible, the weakest link in many countries will still be the availability of proper consumption data, especially for infants and children. Therefore, the need for better consumption data was stressed. However, it was recognised that this was not only a problem in the assessment of the trichothecenes, but a general problem in the exposure assessment of food constituents at large.

It was agreed that the use of probabilistic modelling of exposure would be useful and that models to calculate both acute and chronic exposure should be integrated. Harmonisation of methods should be considered, in particular in relation to how to treat subpopulations such as infants and children.

Toxicology

Among the trichothecenes the toxicological database is best for DON, although it is not complete. The less complete databases for NIV, T-2 and HT-2 are reflected in the substantially larger safety factors used by the SCF and JECFA in setting the t-TDIs. It was generally agreed that only more and better toxicological data could reduce the uncertainties in these evaluations and provide the basis for the use of more accurate safety factors. This would not automatically mean that higher TDIs would be established as more intensive toxicological testing might also result in the recognition of more sensitive end-points and lower NOAEL values.

In general, it was agreed that the mechanism behind the neurotoxicity (emesis and feed refusal) needs to be identified. This would allow better decisions to be made on the need for additional studies. A number of additional studies were identified for DON, NIV and T-2/HT-2 in order to reduce the uncertainties in the risk assessment.

DON:

Additional genotoxicity studies and a carcinogenicity study in a second species were proposed in order to complement the toxicological database. There are indications that growth retardation, the critical effect seen in mice, is reversible. This should be further substantiated in 60 or 90 days studies in pigs.

It was also generally accepted that the establishment of an acute reference dose (ARfD) for DON would be very helpful. However, the question was what kind of data should or could be obtained. Human studies would be ideal but the practical and ethical problems were recognised. Instead studies using primates were suggested. Moreover, it was emphasised that for the establishment of an ARfD a 2-4 weeks dosing schedule was needed more than a single-dose study.

NIV:

For NIV, additional genotoxicity studies and a long-term study were required for the establishment of a NOAEL.

T-2/HT-2:

Further studies are needed to reduce the uncertainty in the evaluation of the carcinogenic potential of T-2/HT-2. However, T-2/HT-2 has been assigned a t-TDI (with a safety factor of 500 as there was no evidence of carcinogenicity of T-2 due to a genotoxic mechanism). An additional long-term carcinogenicity study in the rat was proposed. This study was given a higher priority than the proposed second long-term study with DON. Pair-fed controls would be crucial in order to account for the impact of reduced body weight on tumour formation.

To establish a NOAEL for T-2 toxin a longer-term study (60-90 days) in the pig, the most relevant species, is needed. This study might benefit by the inclusion of a reversibility component. An acute NOAEL should also be determined in the pig to establish an acute reference dose.

All trichothecenes:

For all the trichothecenes the following studies were requested:

- studies on neurotoxicity,
- comparative studies of toxicity and toxicokinetics in several species,
- long-term, low dose studies on immunotoxicity and haematotoxicity,
- studies on combined effects

Although studies on combined effects were considered important, studies on potential synergistic actions of different trichothecenes have low priority because they do not seem to occur at the low levels of human exposure. From the discussion, it also appeared that reproductive studies were not a priority because high doses of DON are needed to elicit such effects.

Exposures above the TDI

Intake estimates from 12 European countries have indicated that young children are the population group at the highest risk of intake above the TDI. However, for most countries these intake estimates were very crude. For HT-2 and T-2 toxins most estimated intakes were above the t-TDI, but the majority of data points for occurrence were input as 50% of the limit of detection of the analytical method. Therefore, doubts were expressed that excursions above the TDI are really occurring. The quality of the analytical data and consumption data needs to be improved in order to provide more realistic exposure estimates.

Occasional excursions above the ADI/TDI erode the safety factor but are generally not of toxicological concern. To evaluate the health consequences, if any, of potential excursions above the TDI it is necessary to know the magnitude and duration of the increased intake. This again calls for a refinement of exposure data. In addition, it is important to evaluate the potential reversibility of any effect to be expected as well as the uncertainties involved. It was suggested that historical data from episodes of human intoxications following consumption of contaminated grains should be reviewed in these respects.

It was also suggested that more toxicological studies on these compounds could remove some of the uncertainties involved in the derivation of the TDIs, and hence result in the use of lower safety factors and higher TDI values. While this might be the case for NIV and T-2 and HT-2 toxins it could not be foreseen that increased testing would result in a safety factor for DON of lower than 100. Increased testing might also result in the identification of lower NOAELs.

There seemed to be general agreement that setting up of maximum limits for DON and other trichothecenes in the relevant foodstuffs coupled with increased monitoring would be an effective way to prevent exceeding the TDI. The effect of introduction of risk management measures was clearly shown to be positive in the experience from The Netherlands where a significant decrease in DON occurrence in foods and hence in the intake of DON was reported for the period 2000-2002 compared to 1998-2000.

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