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INTERNATIONAL PROGRAMME ON CHEMICAL SAFETY

Environmental Health Criteria 105

Selected Mycotoxins: Ochratoxins, Trichothecenes, Ergot

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Environmental Health Criteria 105

SELECTED MYCOTOXINS: OCHRATOXINS, TRICHOTHECENES, ERGOT

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The International Programme on Chemical Safety (IPCS) is a joint venture of the United Nations Environment Programme, the International Labour Organisation, and the World Health Organization. The main objective of the IPCS is to carry out and disseminate evaluations of the effects of chemicals on human health and the quality of the environment. Supporting activities include the development of epidemiological, experimental laboratory, and risk-assessment methods that could produce internationally comparable results, and the development of manpower in the field of toxicology. Other activities carried out by the IPCS include the development of know-how for coping with chemical accidents, coordination of laboratory testing and epidemiological studies, and promotion of research on the mechanisms of the biological action of chemicals.

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NOTE TO READERS OF THE CRITERIA DOCUMENTS

Every effort has been made to present information in the criteria documents as accurately as possible without unduly delaying their publication. In the interest of all users of the environmental health criteria documents, readers are kindly requested to communicate any errors that may have occurred to the Manager of the International Programme on Chemical Safety, World Health Organization, Geneva, Switzerland, in order that they may be included in corrigenda, which will appear in subsequent volumes.

* * *

ENVIRONMENTAL HEALTH CRITERIA FOR SELECTED MYCOTOXINS: OCHRATOXINS, TRICHOTHECENES, AND ERGOT

A WHO Task Group on Environmental Health Criteria for Selected Mycotoxins met in London on 14–18 November, 1988. Dr Malcolm HUTTON opened the meeting on behalf of the Director of the Monitoring and Assessment Research Centre (MARC), King's College, London, which hosted the meeting on behalf of the three cooperating organizations of the International Programme on Chemical Safety (WHO/ILO/UNEP). The Task Group reviewed and revised the draft criteria document and made an evaluation of the health risks of exposure to selected mycotoxins.

The draft documents for Ochratoxins and Ergot were prepared by Professor P. KROGH. Those for Trichothecenes were prepared by Dr M. NAKADATE AND HIS COLLEAGUES in the National Institute of Hygienic Sciences and by Professor Y. UENO AND HIS COLLEAGUES in Tokyo. During the task group meeting, several members of the Group agreed to undertake a substantial revision of the draft. Dr A. PROST was responsible for the overall scientific content of the document and Mrs M. O. HEAD of Oxford, England, for the editing.

The Secretariat wishes to acknowledge the contributions of: Dr J. GILBERT (Chemistry and analytical methods for trichothecenes); Dr A.E. POHLAND(Sources and natural occurrence of trichothecenes); and Professor W.W. CARLTON (Animal studies and metabolism of trichothecenes).

The Secretariat also wishes to thank Professor Y. UENO, Co-rapporteur of the Task Group, for his significant contributions and revisions of the draft document during the meeting. Professor H. TANDON, Chairman of the Task Group, and Professor P. KROGH and Professor Y. UENO, Co-rapporteurs, met with members of the Secretariat in Tokyo, 25–30 July, 1989, to review the final document before its release.

The efforts of all who helped in the preparation and finalization of the document, especially those of Dr H. KURATA and Dr M. ICHINOE (National Institute of Hygienic Sciences, Tokyo) and Dr K. OHTSUBO (Tokyo Metropolitan Institute of Gerontology), are gratefully acknowledged.

* * *

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INTRODUCTION

A decade has passed since the publication of Environmental Health Criteria 11: Mycotoxins (WHO, 1979), but this field of research has expanded rapidly, and recent data indicate that the health effects of several of the mycotoxins dealt with in the above publication should be updated. More than 200 mycotoxins are now known to exist. They are present in the environment and, in some cases, human exposure has been documented, mainly through food contamination or occasionally through inhalation. However, information on adverse human health effects is often lacking and, in many cases, the association between exposure to selected mycotoxins and the occurrence of health disorders remains hypothetical.

Ochratoxin A has been found as a contaminant in foods with a frequency in the range of 2–30% in all countries where attempts to perform food analysis have been made. Field cases of ochratoxin A-associated nephropathy in farm animals have been encountered in many countries, underlining the nephrotoxic potential of this compound. More recently, ochratoxin A has been detected in the blood of 6–18% of the human population in some areas where Balkan endemic nephropathy is prevalent. Ochratoxin A has also been found in human blood samples outside the Balkan peninsula. In some studies, more than 50% of the samples analysed have been contaminated. A high incidence of tumours of the urinary system is strongly correlated with the prevalence of Balkan endemic nephropathy. For these reasons, the human health effects of ochratoxin A have been re-evaluated.

A considerable increase in trichothecenes research has been seen over the last decade. The potential for the production of a number of trichothecenes among *Fusarium* species is well documented, and the toxicology of a few trichothecenes and the natural occurrence of some of these compounds in food is fairly well established. Thus, food-borne exposure of human beings to some trichothecenes, in particular deoxynivalenol (vomitoxin) and nivalenol, is likely to occur. Several reports have recently associated outbreaks of human disease with the presence of

trichothecenes in food. For this reason, the health effects of trichothecenes have been re-evaluated.

Ergotism is by far the oldest known mycotoxicosis in man and animals. Recent episodes of *Claviceps purpurea*-associated intoxication in Ethiopia, as well as episodes of *Claviceps fusiformis*-associated intoxications in areas of India indicate that ergotism is still a disease of public health importance, particularly in developing countries. The evaluation of ergot as a food contaminant has therefore been included in the present updated environmental health criteria dealing with selected mycotoxins. However, the review of available documentation has concentrated on studies dealing with the naturally occurring ergot alkaloids. Derivatives produced by the pharmaceutical industry have been deliberately excluded. No attempt has been made to review the literature on the pharmacology of derivatives of lysergic acid. The section on ergot in this publication aims at alerting the scientific community about the present status of ergotism as a disease of our time, and about the differences in clinical symptoms that are observed between Asia and Africa in relation to the chemical differences in responsible toxins.

Studies on the etiology of hepatocellular carcinoma, in particular those indicating that there is a consistent and specific causal association between hepatitis B virus and hepatocellular carcinoma, as well as the existence of other etiological factors that may cause hepatocellular carcinoma independently, have attracted much attention since the publication of the previous EHC on mycotoxins (WHO, 1979). A report by WHO (1983) recognized the value of available methods for the direct assessment of individual exposure to aflatoxins, and the methods and their use in field studies were considered in a later report by IARC (1984). Since the evaluation of the carcinogenic risks of aflatoxins for human beings has recently been reviewed by IARC (1987a), the present update on mycotoxins will not assess the issue and readers should refer to the IARC evaluation.

SUMMARY AND RECOMMENDATIONS FOR FURTHER RESEARCH

1. Ochratoxin A

1.1 Natural occurrence

Ochratoxins are produced by several species of the fungal genera *Aspergillus* and *Penicillium*. These fungi are ubiquitous and the potential for the contamination of foodstuffs and animal feed is widespread. Ochratoxin A, the major compound has been found in a number of countries in Australasia, Europe, and North America. Ochratoxin formation by *Aspergillus* species appears to be limited to conditions of high humidity and temperature, whereas at least some *Penicillium* species may produce ochratoxin at temperatures as low as 5 °C.

The highest incidences of ochratoxin A contamination have been found in cereals, and to a lesser extent in some beans (coffee, soya, cocoa). Ochratoxin B occurs extremely rarely.

1.2 Analytical methods

Analytical techniques have been developed for the identification and quantitative determination of ochratoxin levels in the μg/kg range.

1.3 Metabolism

Residues of unchanged ochratoxin A have been found in the blood, kidney, liver, and muscle of pigs in slaughter houses and in the muscle of hens and chickens. However, residues of ochratoxin A have not generally been found in ruminants. The *in vitro* binding of ochratoxin A to serum albumin is particularly strong in cattle, pigs, and man. Experimental studies on pigs and hens have shown that higher levels of ochratoxin A occur in the kidneys. Microsomal hydroxylation might represent a detoxification reaction in pigs, rats, and man. In experimental studies,

residues could still be identified in pig kidneys, one month after the termination of exposure.

1.4 Effects on animals

Field cases of ochratoxicosis in farm animals (pigs, poultry) have been reported from several European countries, the primary manifestation being chronic nephropathy. The lesions include tubular atrophy, interstitial fibrosis and, at later stages, hyalinized glomeruli. Ochratoxin A has also been found in pig blood collected at Canadian slaughterhouses. It has produced nephrotoxic effects in all species of single-stomach animals studied so far, even at the lowest level tested (200 μg/kg feed in rats and pigs).

Teratogenic effects were observed in mice exposed orally to 3 mg/kg body weight. Fetal resorption was observed in rats given doses from 0.75 mg/kg body weight orally. Teratogenic effects, which in the rat were enhanced by a diet low in protein, have also been observed in hamsters.

There is no evidence of ochratoxin A activity in short-term tests for mutagenicity (bacteria and yeasts). Rats exposed orally showed single-strand breaks in DNA in renal and hepatic tissues. Ochratoxin A induced renal cell neoplasms in male mice and in both sexes of rats dosed orally. Hepatic cell neoplasms were reported in only one mouse strain and not in the rat.

Ochratoxin A is an inhibitor of protein synthesis and tRNA synthetase in microorganisms, hepatoma cells, and in renal mRNA in the rat.

Ochratoxin A can inhibit macrophage migration. In mice, a dose of 0.005 μg/kg body weight suppressed the immune response to sheep erythrocytes; however, contradictory results have also been obtained.

Ochratoxin A has been shown to be carcinogenic to the renal tubular epithelium in male mice and in both sexes in rats.

1.5 Effects on man

Human exposure, as demonstrated by the occurrence of ochratoxin A in food, blood, and in human milk, has been observed in various countries in Europe. Available epidemiological information indicates that Balkan nephropathy may be associated with the consumption of foodstuffs contaminated by this toxin.

A highly significant relationship has been observed between Balkan nephropathy and tumours of the urinary tract, particularly with tumours of the renal pelvis and ureters. However, no data have been published that establish a direct causal role of ochratoxin A in the etiology of such tumours.

2. Trichothecenes

2.1 Natural occurrence

To date, 148 trichothecenes, characterized chemically as having the same basic tetracyclic scirpenol ring system, are known. These compounds are produced primarily by moulds belonging to the genus *Fusarium*, though other genera, including *Trichoderma, Trichothecium, Myrothecium,* and *Stachybotrys*, are also known to produce metabolites now characterized as trichothecenes. Only a few of the known trichothecenes have been found to contaminate food or animal feed including: deoxynivalenol (DON), nivalenol (NIV), diacetoxyscirpenol (DAS), and T-2 toxin and, less frequently, certain derivatives (3-Ac-DON, 15-Ac-DON, fusarenon-X and HT-2 toxin). Of these, by far the most commonly encountered in food and animal feed is DON, with lesser amounts of NIV usually found as co-contaminants. Some macrocyclic trichothecenes, such as satratoxins G and H, and the verrucarins, occur occasionally in animal feed (straw, hay) but there are no reports of their presence in foods.

Surveys for the presence of trichothecenes have revealed the world-wide occurrence of DON, primarily in cereals, such as wheat and corn, at levels occasionally as high as 92 mg/kg, though average levels are considerably lower and vary with commodity. There are isolated reports of the occurrence of DON in barley,

mixed feeds, potatoes, etc. NIV, though not normally reported in cereals in Canada or the USA, is commonly found in conjunction with DON in Asian and European grains; the highest concentration recorded to date for NIV is 37.9 mg/kg. T-2 toxin and DAS have been reported infrequently and at much lower concentrations.

Processing and milling studies have shown little reduction in DON levels from the cereal to the finished product. Similarly, baking is not effective in destroying DON. In general, commercially available human foodstuffs rarely contain detectable levels of DON and NIV.

2.2 Analytical methods

Analytical methods based on TLC, GC, HPLC, and immunological techniques are available for the determination of the four most frequently encountered toxins (DON, T-2 toxin, DAS, NIV) with detection limits below 1 μg/g. Several of these methods have been tested collaboratively. In addition, research methods, such as GC/MS and LC/MS, are available for confirmation of identity.

2.3 Metabolism

Metabolic studies have been carried out on animals, principally with T-2 toxin, but a few with DON. These trichothecenes are rapidly absorbed from the alimentary tract, but quantitative data are not available. The toxins are distributed fairly evenly without marked accumulation in any specific organ or tissue. Trichothecenes are metabolically transformed to less toxic metabolites by such reactions as hydrolysis, hydroxylation, de-epoxidation, and glucuronidation. Trichothecenes, such as T-2 toxin and DON, are rapidly eliminated in the faeces and urine. For example, almost 100% of an oral dose of T-2 toxin in cattle was eliminated within hours of dosing; in chickens, about 80% had been eliminated 48 h after dosing. In the rat, 25% of DON was eliminated in the urine and 65% in the faeces, 96 h after dosing. The results of transmission of T-2 toxin in the laying hen and lactating cow showed that less than 1% of the administered dose of

this toxin and its metabolites was present in eggs and milk. Tissue residues of oral T-2 toxin and metabolites in chicken meat were below 2% of the dose, 24 h after dosing.

2.4 Effects on animals

Ingestion of animal feed of plant origin is the main route of exposure to trichothecenes. T-2 toxin and DAS, which are the most potent for laboratory animals of the trichothecenes commonly reported as feed contaminants (T-2 toxin, DAS, NIV, and DON), induce a similar toxic response. NIV is less potent in some systems than the previous two compounds and DON is the least toxic of the four (examples of potency include the oral LD_{50}s in the mouse: T-2 toxin, 10.5 mg/kg body weight and DON, 46.0 mg/kg).

The more potent trichothecenes, such as T-2 toxin and DAS, produce acute systemic effects when administered experimentally to rodents, pigs, and cattle, via the oral, parenteral, or inhalation (pig, mouse) route. Epithelionecrosis is a lesion produced by contact exposure with potent trichothecenes, such as T-2 toxin and DAS (dose of 0.2 μg per spot for T-2 toxin). Larger doses of other trichothecenes (NIV, 10 μg per spot) are required to produce an irritant effect. The cytotoxic trichothecenes, such as T-2 toxin, produce necrosis of the intestinal crypt epithelium and of lymphoid and haematopoietic tissues after oral, parenteral, or inhalation exposure. Haematological and coagulopathic abnormalities follow exposure to cytotoxic trichothecenes, such as T-2 toxin and DAS. Severe toxicosis can result in pancytopenia. Suppression of cell-mediated and humoral immunity has been demonstrated in studies with T-2 toxin, DON, and DAS, and observations include effects such as reduced concentrations of immunoglobulins and depressed phagocytic activity of both macrophages and neutrophils. The results of experimental animal studies have indicated that the immunodepressive effect of such trichothecenes as T-2, DAS, and DON, results in decreased resistance to secondary infection by bacteria (Mycobacteria, *Listeria monocytogenes*), yeasts (*Cryptococcus neoformans*), and viruses (Herpes simplex virus).

T-2 toxin has been reported to be teratogenic in the mouse, when given by intraperitoneal injection (unusual route of administration for teratogenic studies). DON was reported to be teratogenic in mice after gastric intubation, but was not teratogenic in rats when the toxin was provided in the feed. NIV was not teratogenic in mice. T-2 toxin, DAS, and DON were not mutagenic in an Ames-type assay. T-2 toxin had weak clastogenic activity in some assays. There is no evidence from the published long-term toxicity studies in animals to indicate that T-2 toxin, fusarenon-X, and NIV are tumorigenic in animals. No long-term studies of DON toxicity have been published.

Trichothecenes are toxic for actively dividing cells, such as the intestinal crypt epithelium and the haematopoietic cells. The cytotoxicity has been associated with either impairment of protein synthesis by the binding of the compounds to the ribosomes of eukaryotic cells, or the dysfunction of cellular membranes. Inhibition of protein synthesis has been associated with the induction of labile and regulatory proteins, such as IL-2 in immunocytes. Transport of small molecules is impaired in cell membranes by extremely low concentrations of trichothecenes.

2.5 Effects on man

Ingestion of contaminated foods of plant origin is the main route of exposure to trichothecenes, but other routes have been reported occasionally, such as accidental skin contact amongst laboratory research workers, and airborne trichothecenes in dust.

Reported cases of illness associated with exposure to trichothecenes are scarce and none has been established as being due to trichothecenes. However, a causative role is suggested by the two outbreaks referred to below.

One disease outbreak was reported from China and was associated with the consumption of scabby wheat containing 1.0–40.0 mg DON/kg. The disease was characterized by gastrointestinal symptoms. No deaths occurred in human beings. Swine and chicks fed the leftover cereals were also affected.

An analogous outbreak was reported from India and was associated with consumption of baked bread made from contaminated wheat. The disease was characterized by gastrointestinal symptoms and throat irritation, which developed within 15 minutes to one hour following ingestion of the bread. The following mycotoxins were detected in samples of refined wheat flour used in the preparation of the bread: DON (0.35–8.3 mg/kg), acetyldeoxynivalenol (0.64–2.49 mg/kg), NIV (0.03–0.1 mg/kg) and T-2 toxin (0.5–0.8 mg/kg). However, there was no confirmation of the identity of the detected trichothecenes. The concomitant occurrence of DON and NIV with T-2 toxin is unusual.

Two diseases of historical interest, alimentary toxic aleukia (ATA) in the USSR and scabby wheat toxicosis in Japan and Korea, have been associated with the consumption of grain invaded by *Fusarium* moulds. Some trichothecenes have since been identified under laboratory conditions in fungal cultures of *Fusarium* moulds isolated from grains involved in the incidents. Studies linking ATA and scabby grain toxicosis to trichothecenes exposure could not be made at the time that the disease occurred, because the toxins were not known.

3. Ergot

3.1 Natural occurrence

Ergot is the name given to sclerotia of fungal species within the genus *Claviceps*. Biologically active alkaloids contained in the sclerotia cause the development of toxicoses when the sclerotia are consumed by man and animals through contaminated food or animal feed.

Ergot alkaloids produce two different patterns of diseases, depending on the fungal organism involved (*C. Purpurea, C. fusiformis*) and hence the alkaloids produced. Ergotism, induced by ergotamine-ergocristine alkaloids produced by *C. purpurea*, is characterized predominantly by gangrene of the extremities as well as gastrointestinal symptoms. Intoxication induced by millet contaminated with *C. fusiformis* is mainly characterized by

gastrointestinal symptoms, and is related to clavine alkaloids. There are no signs or symptoms suggesting vaso-occlusion.

3.2 Analytical methods

Ergot alkaloids (ergolines) are derivatives of lysergic acid. The individual alkaloids vary in the magnitude of their biological activity. Determination of *C. purpurea* ergot alkaloids has been carried out by HPLC with fluorescence detection. Concentrations of 0.2 μg ergoline per litre of human plasma can be measured. Ergotamine and ergocristine can be determined very specifically by radioimmunoassays at levels of 3.5 picomoles and 0.8 picomoles, respectively.

3.3 Effects on animals

Ergolines, mainly ergotamine and ergotaminine, have been associated with outbreaks of bovine abortion. Sheep, administered ergotamine orally, rapidly became ill and intestinal inflammation was observed. Orally exposed poultry, pigs, and primates experienced slight effects. No data on the mutagenicity, teratogenicity, and carcinogenicity of ergolines were available to the task group.

3.4 Effects on man

Claviceps-infected grain is a source of human exposure to ergolines. In most toxicological studies, identification of specific alkaloids has not been undertaken. The published information from only one survey of cereals and cereal products indicates a total daily human intake of ergolines in Switzerland of approximately 5.1 μg per person, the contents of certain commodities being up to 140 μg/kg. Baking reduces the ergolines present in contaminated flour by 25–100%.

An outbreak of ergotism in Ethiopia in 1978 resulted from exposure to ergolines from *C. purpurea* sclerotia. The grain contained up to 0.75% ergot; ergometrine was detected specifically. Symptoms included dry gangrene with loss of one or more limb (29% of cases), feeble or absent peripheral pulses (36%), and

desquamation of the skin. Gastrointestinal symptoms occurred in only a few cases. Lower extremities were involved in 88% of patients.

In India, several outbreaks have occurred since 1958 as a result of ingesting pearl millet containing clavine-type ergot from *C. fusiformis*. Symptoms included nausea, vomiting, and giddiness. Pearl millet containing 15–26 mg ergoline/kg caused the toxic symptoms.

Since autopsies were not performed in either of the episodes, no information is on record of the pathological effects on human viscera.

4. Recommendations for further research

4.1 General recommendations

A network of reference centres should be established to assist Member States in confirming the identity of individual mycotoxins found in human foods and tissues. These reference centres should also provide mycotoxin reference samples, upon request, to reinforce the intercomparability of analytical results obtained in different parts of the world.

4.2 Ochratoxin A

(*a*) Extended retrospective, as well as focal prospective, epidemiological studies on the association of ochratoxin A with Balkan type endemic nephropathy and with urinary-tract tumours should be conducted in the Balkan peninsula and the Mediterranean region.

(*b*) Blood analysis for ochratoxin A should be performed on patients with urinary-tract tumours, outside the Balkan peninsula.

(*c*) The source of ochratoxin A exposure, as indicated by human blood analysis, should be elucidated in countries outside the Balkan peninsula.

(*d*) The mechanism of the sex differences in renal, neoplastic, and non-neoplastic disease, caused by ochratoxin A in experimental animals, should be elucidated.

(*e*) Extended surveys on the ochratoxin A contents of foods in different parts of the world are required. Such surveys are particularly important in regions of the world where high incidence rates of urinary-tract tumours, renal tumours, or nephropathy occur.

4.3 Trichothecenes

(*a*) Follow-up studies should be performed in the areas of India and the People's Republic of China in which episodes of trichothecenes intoxication in human beings have been encountered recently. The unusual pattern of trichothecene occurrence in these episodes should be further elucidated.

(*b*) The effects of long-term exposure of experimental animals to DON, including the carcinogenic effects, should be studied. Because the response to DON by different species varies greatly, the test species must be chosen carefully.

(*c*) Secondary microbial infection in experimental animals following trichothecenes exposure should be further elucidated.

(*d*) The influence of environmental conditions, including the presence of insecticides and other man-made chemicals, on the fungal production of trichothecenes should be studied.

(*e*) The effects of food processing on trichothecenes should be clarified.

(*f*) Agricultural plants, resistant to infection by trichothecene-producing fungi, should be developed, using biotechnological approaches.

(*g*) The possible synergistic effects in experimental animals of combined exposure to trichothecenes, aflatoxins, ochratoxin A, and other mycotoxins should be studied.

(*h*) Studies on the intake of trichothecenes by human beings should be performed.

(*i*) Rapid and sensitive screening procedures for trichothecenes should be developed, and surveys for trichothecenes in grain and processed foods in temperate zones of the world should be conducted.

4.4 Ergot

(*a*) Methods of analysis for agroclavines should be developed.

(*b*) Information should be made available to developing countries on the use of pathological seed screening and milling procedures for minimizing the problems of ergot.

(*c*) Epidemiological studies should be performed on the possible effects of low levels of ergolines on the human population.

(*d*) Pharmacological and toxicological studies should be performed using individual and combined ergolines on experimental animals.

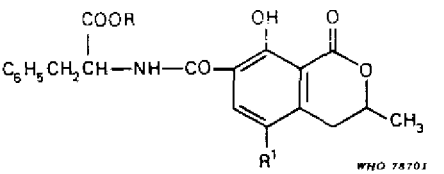
(*e*) The possible transmission of ergolines through the mother's milk to the infant should be elucidated.

I. OCHRATOXINS

I.1 PROPERTIES AND ANALYTICAL METHODS

I.1.1 Chemical properties

The ochratoxins constitute a group of closely-related derivatives of isocoumarin linked to L-phenylalanine (Fig. 1), and classified according to biosynthetic origin as pentaketides within the group of polyketides (Turner, 1971). The topic has been reviewed by Scott (1977) and Steyn (1977, 1984).



The chemical structure of ochratoxins: ochratoxin A: R^1 = Cl, R = H; ochratoxin B: R^1 = H, R = H; ochratoxin C: R^1 = Cl, R = C_2H_5; methyl ester of ochratoxin A: R^1 = Cl, R = CH_3; methyl or ethyl ester of ochratoxin B: R^1 = H, R = CH_3 or C_2H_5.

Fig. 1. The chemical structure of ochratoxins.

The first compound discovered, ochratoxin A, was isolated from a culture of *Aspergillus ochraceus,* hence the name (Van der Merwe et al., 1965). The acids, including 4-hydroxy-ochratoxin A, the methyl and ethyl esters, and the isocoumarin part of ochratoxin B (ochratoxin) have all been isolated from fungal cultures under experimental conditions. On acid hydrolysis, ochratoxin A yields phenylalanine, and the isocoumarin part, ochratoxin α, a cleavage product also found in the intestines, faeces, urine, and liver of rodents experimentally fed an ochratoxin A-containing diet (Galtier & Alvinerie, 1976). Ochratoxin A and, very rarely,

ochratoxin B are the only compounds found as natural contaminants in plant material, and most of the information available concerns ochratoxin A. It can be stored in ethanol in the refrigerator for more than a year without loss (Chu & Butz, 1970); however, such solutions should be protected from light, since decomposition occurs on exposure to fluorescent light for several days (Neely & West, 1972).

Ochratoxin A is a colourless, crystalline compound, obtained by crystallization from benzene, with a melting point of about 90 °C, and containing approximately one mole of benzene. After drying for 1 h at 60 °C, it has a melting point in the range of 168–173 °C. It is soluble in polar organic solvents, slightly soluble in water, and soluble in dilute aqueous bicarbonate. Physical data on ochratoxin A, based on a collaborative study, have been published by IUPAC (Pohland et al., 1982). Ochratoxin A is optically active: $[\alpha]_D^{21}$: −46.8 °C (c = 2650 μmol/litre in chloroform). The publication cited above includes information on the following spectra of ochratoxin A: ultraviolet absorption spectrum; infrared absorption spectrum; electron impact mass spectrum; nuclear magnetic resonance spectrum.

I.1.2 Methods for the analysis of foodstuffs and biological samples

Ochratoxin A in acidified commodities is readily soluble in many organic solvents, and this characteristic has been used as the principle of extraction in several methods.

A number of methods using thin-layer chromatography have been published, one of the most commonly used being the official AOAC method developed for barley (Nesheim et al., 1973). In this method, ochratoxins A and B are extracted from ground samples with chloroform, after acidification. The toxins are trapped in a column containing diatomaceous earth impregnated with a basic aqueous solution. After column clean-up, the toxins are eluted and thin-layer chromatography is performed using long-wave UV irradiation for visualization of the fluorescent ochratoxin spots (limit of detection: 12 μg/kg). The method has been collaboratively studied, revealing coeffecients of variation (between laboratories) in the range of 31–54% (Nesheim, 1973).

This method has been published as an IUPAC recommended procedure (IUPAC, 1976). The sensitivity can be improved by exposing the developed plate to ammonia fumes, resulting in a limit of detection of a few μg/kg. A slightly modified method, developed for green coffee (Levi, 1975), has a coefficient of variation in the range of 33–49%, based on a collaborative study.

The two methods have been combined into one procedure, published as an IARC procedure (Nesheim, 1982). In an international check sample survey on ochratoxin A in animal feed, the AOAC procedure was used by 61% of the participants, and the performance was slightly better than that of the other methods, with a coefficient of variation of 69% compared to 79% for the other methods combined (Freisen & Garren, 1983). Paulsch et al. (1982) developed a procedure for the determination of ochratoxin A in the kidneys of swine, using a liquid–liquid partitioning step instead of column clean-up, followed by two-dimensional thin-layer chromatography using an acidic and an alkaline developing solvent. In addition, the procedure included a confirmatory test, based on the formation of ochratoxin A methyl ester on the plate.

High-performance liquid chromatographic procedures have been developed for the determination of ochratoxin A in food of plant origin (Hunt et al., 1978; Josefsson & Moller, 1979), with limits of detection in the range of 1–12 μg/kg, and with recoveries in the 55–92% range. A procedure for the determination of ochratoxin A residues in renal tissue has been published, in which enzymic digestion of the sample tissue is followed by dialysis and high performance liquid chromatography (Hunt et al., 1979). The limit of detection is about 1 μg/kg, and recoveries of 77–78% have been observed. Rapid screening methods for ochratoxin A are available, based on minicolumn chromatography, with limits of detection in the 8–12 μg/kg range (Hald & Krogh, 1975; Holaday, 1976). By using antisera to ochratoxin A, an enzyme-linked immunosorbent assay (ELISA) has been constructed in which the toxin in barley can be determined with only 0.5% cross-reaction for ochratoxin B, and with a lower level of detection of 10 pg ochratoxin A/well of the ml plate (Morgan et al., 1982). Immunological methodology has been further improved by the use of monoclonal antibody (IgG) in a radio-

immunoassay with high specificity for ochratoxin A, which can detect levels of this toxin as low as 0.2 μg/kg in swine kidney tissue (Candlish et al., 1986; Rousseau et al., 1987). A method has been described in which ochratoxin A is cleaved to ochratoxin α and phenylalanine using the enzyme carboxypeptidase. The quantification of ochratoxin A is based on the loss of fluorescence intensity at 380 nm, the limit of detection being 4 μg/kg of barley (Hult & Gatenbeck, 1976). The procedure has also been applied to swine blood with a limit of detection of 2 μg/litre (Hult et al., 1979).

The same procedure has been used in screening human blood for the presence of ochratoxin A, with a limit of detection of 1–2 μg/litre serum for a 2-g sample. High-performance liquid chromatography was used as confirmation (Hult et al., 1982). A screening method involving flow injection has been developed by which ochratoxin A concentrations of more than 10 μg/litre can be determined, based on 50-μlitre samples of serum (Hult et al., 1984). As the specificity of the method is limited, positive results have to be confirmed by conventional methods requiring much larger blood samples.

A method has been developed for the detection of ochratoxin A (and aflatoxin B and citrinin) in human urine using hydrolysis of urine, solid-phase extraction, and reversed-phase liquid chromatography with fluorescence detection (Orti et al., 1986). The detection limit for ochratoxin A was approximately 10 ng/ml in samples of 10 ml urine.

I.2 SOURCES AND OCCURRENCE

I.2.1 Fungal formation

The ochratoxins were isolated in 1965 from a culture of *Aspergillus ochraceus*, hence the name (Van der Merwe et al., 1965), but subsequent investigations have revealed that a variety of fungal organisms included in the genera *Aspergillus* and *Penicillium* are able to produce ochratoxins (Table 1).

Table 1. Ochratoxin-producing fungi[a]

Penicillium link	
Monoverticillata:	
P. frequentans series:	*P. purpurrescens* Sopp
Asymmetrica lanata:	
P. commune series:	*P. commune* Thom
Asymmetrica fasciculata:	
P. viridicatum series:	*P. viridicatum* Westling
	P. palitans Westling
P. cyclopium series:	*P. cyclopium* Westling
Biverticillata symmetrica:	
P. purpurogenum series:	*P. varibile* Sopp
Aspergillus Micheli	
Aspergillus ochraceus group:	*A. sulphureus* (Fres.) Thom & Church
A. sclerotiorum Huber	
A. alliaceus Thom & Church	
A. melleus Yukawa	
A. ochraceus Wilhelm	
A. ostianus Wehmer	
A. petrakii Vörös	

[a] From: Krogh (1978).

The effects of water activity (a_w) and temperature, the main factors controlling mycotoxin formation, have been elucidated in relation to growth and ochratoxin production for 3 fungal organisms: *A. ochraceus*, *P. cyclopium*, and *P. viridicatum* (Northolt et al., 1979). The minimum a_w values for ochratoxin production ranged between 0.83 and 0.87, 0.87 and 0.90, and 0.83

and 0.86, respectively. At 24 °C, optimum a_w values for *A. ochraceus* and for both *P. cyclopium* and *P. viridicatum* were 0.99 and 0.95–0.99, respectively (Fig.2).

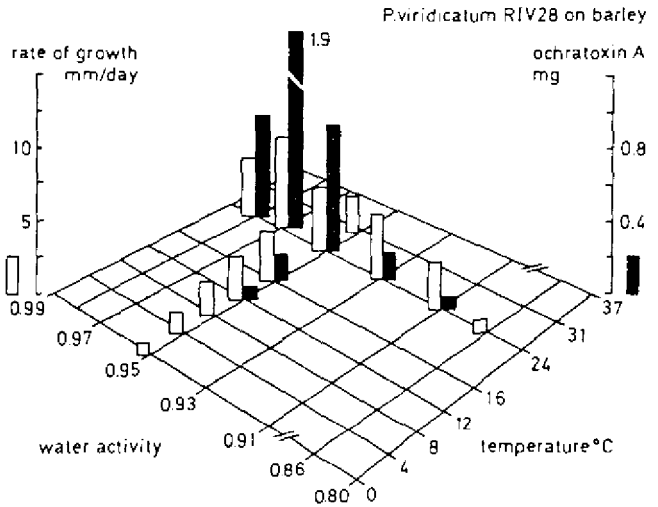


Fig. 2. Growth of, and ochratoxin A production by, *Penicillium viridicatum* RIV 28 on barley meal under various conditions of water activity and temperature (the 1 mm black columns represent amounts of 1-40 μg of ochratoxin A).

At optimum a_w, the temperature range for ochratoxin production by *A. ochraceus* was 12–37 °C, whereas that of *P. cyclopium* and *P. viridicatum* was 4–31 °C. These laboratory data correspond with those from field observations on ochratoxin contamination in crops. Thus, the more frigophilic *Penicillia*, particularly *P. viridicatum*, are the major ochratoxin producers in crops in colder climatic zones, such as Scandinavia (Krogh, 1978; Rutqvist et al., 1978; Häggblom, 1982), and in Canada (Scott et al., 1972).

In contrast, ochratoxin-producing fungal potential has been found in 28–50% of *A. ochraceus* strains isolated from crops in warmer climatic zones, such as Australia (Connole et al., 1981),

and Yugoslavia (Pepeljnjak & Cvetnic, 1981),and strains isolated from coffee beans (Stack et al., 1982).

I.2.2 Occurrence in foodstuffs

I.2.2.1 Plant products

Ochratoxin A was first encountered as a natural contaminant in maize (Shotwell et al., 1969), and subsequent surveys have established that ochratoxin A is a contaminant of cereals and some beans (coffee beans, soya beans, cocoa beans) in many areas of the world (Table 2). Although the mean level of ochratoxin A in all reported surveys up to 1979 was 1035 μg/kg (Fig. 3), 83% of the samples contained less than 200 μg/kg (Krogh, 1980).

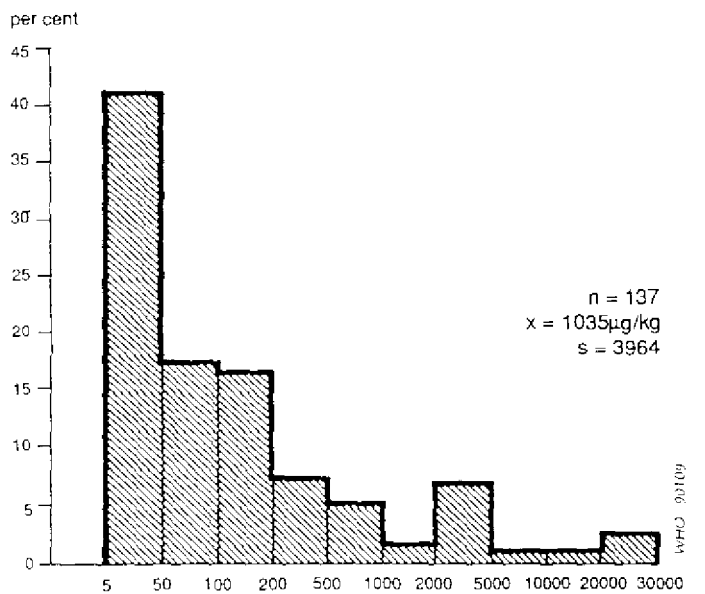


Fig. 3. Frequency of ochratoxin A contamination levels, based on all reported surveys of foodstuffs up to 1979. *Ordinate*: μg ochratoxin A/kg; *Abscissa*: percentage of contaminated samples (From Krogh, 1980).

Table 2. Natural occurrence of ochratoxin A in foodstuffs and animal feed of plant origin

Commodity	Country	Number of samples analysed	Percentage contaminated	Range of ochratoxin A levels (μg/kg)	Reference
FOOD					
maize	USA	293	1.0	83–166	Shottwell et al. (1971)
maize (1973)	France	463	2.6	15–200	Galtier et al. (1977b)
maize (1974)	France	461	1.3	20–200	Galtier et al. (1977b)
wheat (red winter)	USA	291	1.0	5–115	Shottwell et al (1976)
wheat (red spring)	USA	286	2.8	5–115	Shottwell et al (1976)
barley (malt)	Denmark	50	6.0	9–189	Krogh (1978)
barley	USA	127	14.2	10–40	Nesheim (1971)
coffee beans	USA	267	7.1	20–360	Levi et al. (1974)
maize	Yugoslavia[a]	542	8.3[b]	6–140	Pavlovic et al. (1979)
wheat	Yugoslavia[a]	130	8.5[b]	14–135	Pavlovic et al. (1979)
wheat bread	Yugoslavia[a]	32	18.8[b]		Pavlovic et al. (1979)
barley	Yugoslavia[a]	64	12.5[b]	14–27	Pavlovic et al. (1979)
barley	Czechoslovakia	48	2.1	3800	Vesela et al. (1978)
bread	United Kingdom[c]	50	2.0	210	Osborne (1980)
flour	United Kingdom[c]	7	28.5	490–2900	Osborne (1980)
beans	Sweden	71	8.5	10–442	Akerstrand & Josefsson (1979)
peas	Sweden	72	2.8	10	Akerstrand & Josefsson (1979)
maize	United Kingdom	29	37.9	50–500	Ministry of Agriculture (1980)
cornflour	United Kingdom	13	30.8	50–200	Ministry of Agriculture (1980)

Table 2 (*continued*)

FOOD (*continued*)					
soya bean	United Kingdom	25	36.0	50–500	Ministry of Agriculture (1980)
soya flour	United Kingdom	21	19.0	50–500	Ministry of Agriculture (1980)
cocoa beans (raw)	United Kingdom	56	17.9	100–500	Ministry of Agriculture (1980)
(roasted)	United Kingdom	19	15.8	100	Ministry of Agriculture (1980)
grain	German Democratic Republic	49	4.1	18–22	Fritz et al. (1979)
grain (barley, wheat, rye)	Poland	296	6.8	20–470	Szebiotko et al. (1981)
grain (wheat, rye)	Denmark	151	1.3	15–50	Pedersen & Hansen (1981)
bran (wheat)	Denmark	57	10.5	5–20	Pedersen & Hansen (1981)
maize	Bulgaria[a]	22	27.3	25–35	Petkova-Bocharova & Castegnaro (1985)
maize	Bulgaria	22	9.0	10–25	Petkova-Bocharova & Castegnaro (1985)
beans	Bulgaria[a]	24	16.7	25–27	Petkova-Bocharova & Castegnaro (1985)
beans	Bulgaria[a]	28	7.1	25–50	Petkova-Bocharova & Castegnaro (1985)
barley, wheat oats, rye, maize	Poland	150	5.3	50–200	Juszkiewicz & Piskorska-Pliszczynska (1976)

Table 2 (*continued*)

Commodity	Country	Number of samples analysed	Percentage contaminated	Range of ochratoxin A level (μg/kg)	Reference
FEED (*continued*)					
mixed feed	Poland	203	4.9	10–50	Juszkiewicz & Piskorska-Pliszczynska (1977)
maize	Yugoslavia	191	25.7	45–5125	Balzer et al. (1977)
barley, oats	Sweden	84	8.3	16–409	Krogh et al. (1974b)
wheat, hay	Canada	95	7.4	30–6000	Prior (1976)
wheat, oats barley, rye	Canada [c]	32	56.3	30–27 000	Scott et al. (1972)
barley, oats	Denmark [c]	33	57.6	28–27 500	Krogh et al. (1973)
mixed feed	Canada	474	1.1	30–100	Prior (1981)
mixed feed	Canada [c]	51	7.8	48–5900	Abramson et al. (1983)
mixed feed	Australia	25	4.0	70 000	Connole et al. (1981)
mouldy bread	Italy [d]	1		80 000	Visconti & Bottalico (1983)

[a] From an area with endemic nephropathy.
[b] Average values for a period of 2–5 years.
[c] All samples suspected of containing mycotoxins.
[d] This sample contained in addition 9600 μg ochratoxin B/kg.

Ochratoxin B has only been found in 3 samples and, thus, occurs extremely rarely; the other ochratoxins have never been found in plant products. Levels of ochratoxin A and the frequency of contamination are generally higher in animal feed than in foodstuffs (Table 2). Although the mean level of ochratoxin A in all reported surveys up to 1979 was 1035 μg/kg (Fig. 3), 83% of the samples contained less than 200 μg/kg (Krogh, 1980).

I.2.2.2 Residues in food of animal origin

Residues of ochratoxin A are not generally found in ruminants, because ochratoxin A is cleaved in the forestomachs by protozoan and bacterial enzymes (Galtier & Alvinerie, 1976; Hult et al., 1976; Patterson et al., 1981). The non-toxic cleavage product, ochratoxin α, has been found in the kidneys at levels below 10 μg/kg and in the blood of calves fed a diet containing 300–500 μg ochratoxin A/kg (Patterson et al., 1981). In half of the calves, the kidneys contained low levels of ochratoxin A (up to 5 μg/kg), a feature that may reflect that the calves were not yet functioning as ruminants. When 2 milking cows were fed a ration containing 317–1125 μg ochratoxin A/kg for 11 weeks, a residue of 5 μg ochratoxin A/kg was found in the kidneys of one of the animals but not in any other tissue or the milk. Ochratoxin α was not found in any tissue (Shreeve et al., 1979). Residues of ochratoxin A have been detected in a number of tissues in single-stomach food animals, such as pigs. The carry-over of ochratoxin A from feed to animal tissues was elucidated in a study in which groups of pigs were exposed for 3-4 months to dietary levels of ochratoxin A of 200, 1000, or 4000 μg/kg (Krogh et al., 1974a). At termination (slaughter), the highest levels of ochratoxin A residues were found in the kidneys (mean levels 50 μg/kg at the 4000 μg/kg feed level), with lower levels in the liver, muscle, and adipose tissue; other tissues, including blood, were not analysed. There was a high correlation between the feed level of ochratoxin A and the residue levels in the 4 tissues investigated (Table 3).

Table 3. Correlation between feed level and tissue levels (residues) of ochratoxin A in pigs[a]

Tissue	Regression equation	*r*
kidney	$Y = 2.15 + 0.0123X$	0.86
liver	$Y = 0.35 + 0.0095X$	0.82
adipose	$Y = 2.51 + 0.0099X$	0.78

[a] Modified from: Krogh et al. (1974a).
X = ochratoxin A in feed (μg/kg).
Y = ochratoxin A residue (μg/kg tissue).
r = correlation coefficient.
The regression is calculated on feed levels of ochratoxin A in the range of 200–4000 μg/kg.

Ochratoxin A is present in the blood bound to serum-albumin (Chu, 1971; Galtier, 1974a) and as free ochratoxin A; saturation (in the rat) occurs at 70 mg ochratoxin A/litre plasma. The binding of ochratoxin A to serum-albumin is particularly strong in cattle, pigs, and man, based on *in vitro* studies (Table 4).

The presence of ochratoxin A in the blood of pigs has been elucidated in experimental animal studies as well as by surveys of blood samples from pigs at farms (Hult et al., 1979, 1980). A total of 1200 pig blood samples, collected from slaughterhouses over various periods during 1986 in western Canada, was screened for the presence of ochratoxin A using an HPLC procedure (Marqhardt et al., 1988). It was shown that 3.6–4.2% of the blood samples contained the toxin at concentrations higher than 20 ng/ml. It appears that the concentration of ochratoxin A in the blood is higher than that in any other tissue. In feeding studies, bacon pigs were exposed for various periods to rations containing ochratoxin A concentrations in the range of 58–1878 μg/kg; blood, kidney, liver, muscle, and adipose

Table 4. *In vitro* binding of ochratoxin A to serum-albumin in several species[a]

Animal species	Number of binding sites	Intrinsic Association constant (M-1)
Cattle	0.58	94 600
Pig	0.56	71 100
Man	0.58	63 500
Horse	0.57	57 400
Chicken	0.51	52 700
Rat	0.68	40 100
Sheep	0.88	22 600

[a] Adapted from: Galtier (1979).

tissues were analysed, at slaughter, for residues of ochratoxin A (Mortensen et al., 1983). The statistical association between residue levels in various tissues is indicated by the regression analyses (Table 5).

Epidemiological studies on the basis of data from meat inspection in Danish slaughterhouses revealed prevalence rates of porcine nephropathy ranging from 10 to 80 cases per 100 000 slaughtered pigs (Krogh, 1976a). Surveys in a number of European countries for residues of ochratoxin A in kidneys from cases of porcine nephropathy revealed that 25–39% of the cases contained ochratoxin A levels in the range 2–100 μg/kg (Table 6).

Ochratoxin A levels of up to 29 μg/kg were found in the muscle of hens and chickens collected at one slaughterhouse (Elling et al., 1975). The birds had been condemned because of nephropathy. In another study, groups of hens were exposed for

Table 5. Regression between residues of ochratoxin A in the serum and certain other tissues[a]

Tissue	Regression equation	r	s_b
kidney	$Y = 0.0651X$	0.89	0.002
muscle	$Y = 0.0346X$	0.88	0.001
liver	$Y = 0.0259X$	0.89	0.001
adipose	$Y = 0.0191X$	0.84	0.001

[a] Adapted from: Mortensen et al. (1983).
X = µg ochratoxin A/litre serum.
Y = µg ochratoxin A/kg in the other tissues.
r = correlation coefficient.
s_b = Standard error of slope.

Table 6. Surveys for ochratoxin A residues in kidneys from cases of porcine nephropathy, based on meat inspection data

Country	No. of porcine nephropathy kidneys investigated	Percentage containing residues of ochratoxin A	Range of ochratoxin A residues (µg/kg wet weight)	Reference
Denmark	60	35	2-68	Krogh (1977)
Germany, Federal Republic of	104	21	0.1-1.8	Bauer et al. (1984)
Hungary	122	39	2-100	Sandor et al. (1982)
Poland	113	24	1-23	Golinski et al. (1984)
Sweden	129	25	2-104	Rutqvist et al. (1977)
Sweden	90	27	2-88	Josefsson (1979)

1–2 years to dietary levels of ochratoxin A of 0.3 or 1 mg/kg (Krogh et al., 1976b). The kidneys contained the highest residues with a mean value of 19 μg/kg tissue in the group fed 1 mg ochratoxin A/kg; the liver and muscle contained lower levels of residues and no ochratoxin A was found in the eggs. These results are in accordance with those of subsequent experimental animal studies. For example, in a study in which 4 groups of hens were fed diets containing 0, 0.5, 1, or 4 mg ochratoxin A/kg, the kidneys contained the highest level of ochratoxin A residues (31 μg/kg) in the highest dose group; eggs were not analysed (Prior & Sisodia, 1978).

In another study, hens were fed 1 mg ochratoxin A/kg feed for 8 weeks; the kidneys contained 3–10 μg ochratoxin A/kg and the liver, 1.5–2.5 μg/kg. The eggs were not analysed (Reichmann et al., 1982). When groups of hens were fed diets containing 2.5 or 10 mg/kg, the kidneys contained 1–6 μg ochratoxin A/kg, lower levels being found in the plasma, muscle, and liver (Juszkiewicz et al., 1982). Eggs from the high-dose group contained 0.7–1.3 μg ochratoxin A/kg, but none was detected in eggs from the low-dose group.

White Leghorn hens (54 birds), divided into four groups, were fed diets for one month containing the following concentrations of ochratoxin A (mg/kg): 0, 1.3, 2.6, and 5.2 (Bauer et al., 1988). At termination, three tissues were analysed for residues. The following ranges of dose-dependent residues were found: serum, 4.7–11.7 ng/ml, liver, 9.1–18.0 ng/g, and yolk, 1.6–4.0 ng/g; very little ochratoxin A was found in the egg white, and none was detected in the tissues of the control group.

I.3 METABOLISM

I.3.1 Absorption

In a study on rats exposed by gavage to a single dose of ochratoxin A at 10 mg/kg body weight, Galtier (1974b) found the highest tissue level of unchanged ochratoxin A in the stomach wall during the first 4 h following administration. The small and large intestine and caecum contained small amounts of unchanged ochratoxin A, and it was concluded that ochratoxin A was absorbed mainly in the stomach. Small amounts (1–3% of the total dose) were detected in the caecum and the large intestine, as the isocoumarin moiety (ochratoxin α), most likely as the result of the hydrolysing action of the intestinal microflora (Galtier & Alvinerie, 1976; Hult et al., 1976).

In a study on intestinal absorption using the same animal species, Kumagai & Aibara (1982) came to the conclusion that the site of maximal absorption of ochratoxin A was the proximal jejunum, and that the portal vein was the primary route of transport from the intestinal tract, though part of the transportation took place through the lymphatics.

Using a highly specific antibody against ochratoxin A, which is used in a peroxidase-antiperoxidase detection method, Lee et al. (1984) studied absorption and tissue distribution in Swiss mice over a 48–h period, after the administration of a single dose of 25 mg ochratoxin A/kg body weight. Ochratoxin A was found in large amounts, indicated by staining, in epithelial cells as well as in macrophages of the lamina propria in the duodenum. Smaller amounts were found in jejunal epithelial cells and lamina propria, and much smaller amounts in the epithelial cells in the esophagus and stomach; no toxin was found in the ileum. These findings suggest that absorption mainly takes place in the duodenum and the jejunum.

I.3.2 Tissue distribution

I.3.2.1 Animal studies

In slaughterhouse cases of mycotoxic porcine nephropathy studied by Hald & Krogh (1972), residues of unchanged ochratoxin A were found in all tissues investigated (kidney, liver, and muscle), the highest level (up to 67 μg/kg) occurring in the kidney. In experimental studies on pigs ingesting feed containing ochratoxin A, residues of the toxin were found in all 4 tissues in the decreasing order of kidney, liver, muscle, adipose tissue (Krogh et al., 1974a). A subsequent study revealed that the concentration of ochratoxin A residues in the blood of the pig was higher than those in the other tissues mentioned above (Mortensen et al., 1983). When rats were exposed orally to an ochratoxin A dose of 10 mg/kg body weight, Galtier (1974b) recovered 0.3% of the administered dose in the whole kidneys, 0.9% in the whole liver, and 0.6% in the total muscle tissue, 96 h after exposure. Chang & Chu (1977), using a single intraperitoneal injection of 1 mg ochratoxin A per rat (labelled with ^{14}C in phenylalanine), found that the kidney contained twice as much unchanged ochratoxin A as the liver after 30 minutes, amounting to 4–5% of the total dose.

In the study by Lee et al. (1984), the largest amounts of ochratoxin A found in the kidney (as indicated by staining intensity) were in the epithelium of the proximal convoluted tubules, and to a lesser extent in the distal convoluted tubules, the descending loop of Henle, and glomeruli and Bowman's capsule. Small amounts were found in hepatocytes as well as in the lumina of bile ducts, but not in biliary epithelium, indicating biliary excretion.

Using pig renal cortical slices, it was found that ochratoxin A enters the proximal tubule cells by the common organic anion transport system (Friis et al., 1988). Ochratoxin A inhibited *p*-aminohippurate (PAH) and phenolsulfophthalein uptake in a dose-dependent manner.

I.3.2.2 Studies on man

In a study in Yugoslavia near Slavonski Brod, where endemic nephropathy is prevalent, 639 samples of serum from the inhabitants of 2 villages were screened for the presence of ochratoxin A; 42 (6.6%) were positive for the toxin "with concentrations in the range of 1–57 ng ochratoxin A/g" (Hult et al., 1982). Detection was carried out using the enzymic spectrofluorometric procedure, and positives were confirmed by the esterification of ochratoxin A in serum and of ochratoxin α obtained from the enzymetic hydrolisates; the esters were measured by high performance liquid chromatography.

In a screening of serum samples in Poland using the same method of analysis, 77 out of 1065 samples (7.2%) contained ochratoxin A, with a mean concentration of 0.27 ng/ml and a maximum value of 40 ng/ml (Colinski, 1987). In the Federal Republic of Germany, 173 out of 306 serum samples (56.6%) contained ochratoxin A, as measured by an HPLC procedure, with a mean of 0.6 ng/g and a range of 0.1–14.4 ng/g (Bauer & Gareis, 1987). Three out of 46 kidneys (6.6%) contained ochratoxin A, with a mean of 0.2 ng/g and a range of 0.1–0.3 ng/g. In a study of blood plasma in Denmark, 46 out of 96 samples (47.9%) contained ochratoxin A, as measured by an HPLC procedure, with a mean of 1.7 ng/g and a range of 0.1–9.2 ng/g (Hald, 1989). In Bulgaria, where endemic nephropathy also occurs in some areas, 45 out of 312 blood samples contained ochratoxin A (14.4%), with a mean of approximately 14 ng/g (Petkova-Bocharova et al., 1988).

I.3.3 Metabolic transformation

It has been shown from *in vitro* studies that ochratoxin A binds to serum-albumin (Chu, 1971, 1974b); this binding has also been observed in *in vivo* studies on rats (Galtier, 1974a; Chang & Chu, 1977). Ochratoxin α has been detected in the urine and faeces of rats injected intraperitoneally with ochratoxin A (Nel & Purchase, 1968; Chang & Chu, 1977), indicating the cleavage of ochratoxin A to ochratoxin α and phenylalanine, under these conditions.

Studies with ^{14}C-labelled ochratoxin A indicated that some other, not yet identified, metabolites are formed in the body. Less than half of the radioactivity excreted in rat urine within 24 h of a single intraperitoneal injection of ^{14}C-phenylalanine-labelled ochratoxin A was identified as ochratoxin A (Chang & Chu, 1977).

In both albino rats and brown rats given ochratoxin A orally or intraperitoneally, 1–1.5% of the dose was excreted as (4R)-4-hydroxyochratoxin A and 25–27% as ochratoxin α in the urine (Storen et al., 1982). In *in vitro* studies using liver microsomes from the pig, rat, and man, both (4R)-and (4S)-4-hydroxyochratoxin A were produced in a hydroxylation process involving cytochrome P-450 (Stormer & Pedersen, 1980; Stormer et al., 1981). 4-Hydroxyochratoxin A is non-toxic for rats in amounts up to 40 mg/kg body weight (Hutchison et al., 1971); thus, it has been concluded that the microsomal hydroxylation most likely represents a detoxification reaction.

1.3.4 Excretion

The results of studies in which ^{14}C-labelled ochratoxin A was injected intraperitoneally in rats demonstrated that the toxin was excreted primarily in the urine (Chang & Chu, 1977), though faecal elimination also occurred to some extent (Galtier, 1974b; Chang & Chu, 1977). In a study in which ^{14}C-labelled ochratoxin A was given orally to rats as a single dose (15 mg/kg body weight), the cumulative elimination after 120 h was 11% ochratoxin A and 23% ochratoxin α in the faeces; 11% ochratoxin A and 12% ochratoxin α in the urine; and 33% ochratoxin A in the bile (Suzuki et al., 1977). Absorption was influenced by enteritis, which was caused by the high ochratoxin A doses given (75% of the LD_{50} value). Ochratoxin A injected intravenously as a single dose (4.1 mg/kg) in albumin-deficient and normal rats was excreted in the bile and urine 20–70 times faster in the albumin-deficient rats than in normal rats, indicating that the binding of ochratoxin A to blood albumin delays the excretion of the compound through the liver and kidney (Kumagai, 1985).

In rats given unlabelled ochratoxin A orally, Storen et al. (1982) found 6% ochratoxin A, 1.5% (4R)-4-hydroxyochratoxin A, and

25–27% ochratoxin α in the urine; 12% ochratoxin A and 9% ochratoxin α were found in the faeces.

The excretion of ochratoxin A in the milk was studied in rabbits intravenously injected with 1–4 mg/kg body weight, as single dose (Galtier et al., 1977a). At the highest dose injected, the milk contained 1 mg ochratoxin A/litre; ochratoxin α and 4-hydroxy-ochratoxin A were not detected. Goats were given a single dose of tritium-labelled ochratoxin A (0.5 mg/kg) and the cumulative excretion (in terms of radioactivity) after 7 days amounted to 53% in the faeces, 38% in the urine, 6% in the milk, and 2% in the serum (Nip & Chu, 1979). Only a small fraction of the radio-activity in milk was ochratoxin A, amounting to 0.026% of the total ochratoxin A given.

In the Federal Republic of Germany, a study of human milk obtained from women in two hospitals (patient category not stated) revealed that 4 out of 36 samples (11.1%) contained ochratoxin A, with a mean value of 0.024 ng/ml and a range of 0.017–0.030 ng/ml (Bauer & Gareis, 1987; Gareis et al., 1988).

In mice given ^{14}C-labelled ochratoxin A intravenously at various stages of pregnancy, the toxin was shown to cross the placental barrier on day 9 of pregnancy, at which time it is most effective in producing fetal malformations (Appelgren & Arora, 1983). The highest toxin concentration was found in the bile, which contained 5 times as much as the blood.

Ochratoxin A has been detected in the urine of bacon pigs suffering from nephropathy (Krogh, unpublished information communicated to the Task Group).

In a study on the disappearance rates for various tissues, female bacon pigs were fed ochratoxin A at a level of 1 mg/kg feed for one month and then kept on a toxin-free diet for another month, during which animals were sacrificed at regular intervals (Krogh et al., 1976a). Ochratoxin A disappeared exponentially (Table 7) from the 4 tissues investigated (kidney, liver, muscle, and adipose tissue) with residual life values (RL_{50})[a] in the range of

[a] RL_{50} = half residual life, calculated from the exponential equations shown in Table 7.

Table 7. The rate of disappearance of ochratoxin A residues from pig tissues after termination of a one-month exposure to ochratoxin A at 1 mg/kg feed[a]

Tissue	Ochratoxin A (μg/kg tissue) at time t (days) after termination of exposure
kidney	$28.22 \exp(-0.1522t)$
liver	$19.49 \exp(-0.1598t)$
muscle	$12.94 \exp(-0.2096t)$
adipose	$4.62 \exp(-0.0565t)$

[a] From: Krogh et al. (1976a).

3.5–4.5 days; the toxin could still be detected in the kidneys one month after termination of exposure.

No data are available on ochratoxin levels in human urine or faeces.

When the level in the serum is known, the ochratoxin A residues in the four other tissues can be calculated (Table 5).

I.4 EFFECTS ON ANIMALS

I.4.1 Field observations

I.4.1.1 Pigs

The effects of ochratoxins on animals have been reviewed by Krogh (1976a, 1978). Cases of mycotoxic porcine nephropathy have been regularly encountered in studies in Denmark since the disease was first discovered 50 years ago (Larsen, 1928). The disease is endemic in all areas of the country, though unevenly distributed. Prevalence rates in 1971 varied from 0.6 to 65.9 cases per 10 000 pigs, and epidemics encountered in 1963 and 1971 were associated with a high moisture content in the grain caused by unusual climatic conditions (Krogh, 1976b). On the basis of these studies, Krogh (1978) concluded that ochratoxin A is the substance most frequently associated with porcine nephropathy, though other factors, such as citrinin, may also be involved.

A survey on porcine nephropathy was conducted at six slaughterhouses in Sweden during the spring months of 1978 (Josefsson, 1979). A prevalence rate of 4.4 cases per 10 000 pigs was encountered corresponding to the endemic level of prevalence rates in Denmark; 26.7% of nephropathic kidneys contained residues of ochratoxin A. In Hungary, an epidemiological study on porcine nephropathy and the association with ochratoxin A was conducted in 1980–81 covering 4 areas in the country (Sandor et al., 1982). A prevalence rate of 2.0 cases per 10 000 pigs was measured, comparable to endemic prevalence rates in Scandinavia; 39% of nephropathic kidneys contained residues of ochratoxin A. The morphological changes in the kidneys in cases of mycotoxic porcine nephropathy were characterized by degeneration of the proximal tubules followed by atrophy of the tubular epithelium, interstitial fibrosis in the renal cortex, and hyalinization of some glomeruli (Elling & Moller, 1973).

In Poland, surveys for porcine nephropathy in 1983 and 1984 revealed prevalence rates of 4.7–5.7 cases per 10 000 pigs, with 5–55% of the nephropathic kidneys containing detectable residues of ochratoxin A, apparently depending on the season of the year (Golinski et al., 1984, 1985). Porcine nephropathy has been encountered in the Federal Republic of Germany (Bauer et al., 1984) and in Belgium (Rousseau & van Peteghem, 1989), with respectively, 21% and 18% of the affected kidneys containing residues of ochratoxin A, in the range of 0.1–12 ng/g. In Canada, 1200 samples of pig blood, collected at a slaughterhouse, were screened for ochratoxin A using HPLC (Marquardt et al., 1988). Levels exceeding 10 ng/ml (11.3%) were found in 136 samples; detection was confirmed by derivative formation and spectrometry. No kidney examination was conducted, but the ochratoxin A concentrations detected in the blood suggest that nephropathy might have been present in some of the pigs.

1.4.1.2 Poultry

In a preliminary study in Denmark on chickens condemned by meat inspectors because of renal lesions, 4 out of 14 birds (29%) were found to have nephropathy associated with the ingestion of ochratoxin A, as revealed by the presence of residues of ochratoxin A in tissues (Elling et al., 1975). The renal lesions were characterized by degeneration of proximal and distal tubules of both reptilian and mammalian nephrons and interstitial fibrosis.

1.4.2 Experimental animal studies

1.4.2.1 Acute and chronic effects

The acute and chronic effects of ochratoxins on experimental animals have been reviewed by Chu (1974a), Harwig (1974), and Krogh (1976a). Different species vary in their susceptibility to acute poisoning by ochratoxin A with LD_{50} values ranging from 3.4 to 30.3 mg/kg (Table 8). When ochratoxin A was administered orally to rats and guinea-pigs, the female was more sensitive than the male. In rats, the kidney is the target organ,

Table 8. Acute toxicity of ochratoxin A

Animal	LD_{50} (mg/kg body weight)	Route of administration	Reference
mouse (female)	22	intraperitoneal	Sansing et al. (1976)
rat (male)	30.3	oral	Galtier et al. (1974)
rat (female)	21.4	oral	Galtier et al. (1974)
rat (male)	28	oral	Kanizawa et al. (1977)
rat (male)	12.6	intraperitoneal	Galtier et al. (1974)
rat (female)	14.3	intraperitoneal	Galtier et al. (1974)
guinea-pig (male)	9.1	oral	Thacker & Carlton (1977)
guinea-pig (female)	8.1	oral	Thacker & Carlton (1977)
white leghorn	3.4	oral	Prior et al. (1976)
turkey	5.9	oral	Prior et al. (1976)
Japanese quail	16.5	oral	Prior et al. (1976)
rainbow trout	4.7	intraperitoneal	Doster et al. (1972)
beagle dog (male)	9 (total dose)	oral[a]	Szczech et al. (1973a)
pig (female)	6 (total dose)	oral[b]	Szczech et al. (1973b)

[a] All 3 dogs, dosed daily with 3 mg/kg body weight, died within 3 days.

[b] Both pigs receiving 2 mg/kg daily were moribund and killed within 3 days, and both pigs receiving 1 mg/kg daily were moribund and killed within 6 days.

but necrosis of periportal cells in the liver has also been noted during studies on acute effects (Purchase & Theron, 1968).

The lesions observed in field cases of mycotoxic nephropathy have been reproduced by feeding diets containing levels of ochratoxin A identical to those encountered in the naturally contaminated products. In a study by Krogh et al. (1974a), 39 pigs

fed rations containing ochratoxin A, at levels ranging from 200 to 4000 μg/kg, developed nephropathy after 4 months at all levels of exposure. Changes in renal function were characterized by impairment of tubular function, indicated particularly by a decrease in Tm_{PAH}/Cin,[a] and reduced ability to produce concentrated urine. These functional changes corresponded well with the changes in renal structure observed at all exposure levels, including atrophy of the proximal tubules and interstitial cortical fibrosis. Sclerosed glomeruli were also observed in the group receiving the highest dose of ochratoxin A of 4000 μg/kg feed. Changes were not seen in any other organs or tissues.

Kidney damage, identical to naturally occurring porcine nephropathy, was produced in another study by feeding pigs (9 animals) with crystalline ochratoxin A in amounts corresponding to a feed level of 1 mg/kg for 3 months. Significant renal tubular functional impairment as measured by a decrease in Tm_{PAH} Cin was detected after only 5 weeks of ochratoxin exposure (Krogh et al., 1976b). The study was continued for a 2-year period during which the renal impairment aggravated slightly without reaching a state of terminal renal failure (Krogh et al., 1979).

In 2 pigs and 9 rats dosed orally with ochratoxin A (400 and 250 μg/kg body weight, respectively) for 5 days, ochratoxin A was detected in the epithelial cells of the proximal convoluted tubules of the nephron of all animals. The method of detection was immunofluorescence microscopy using an antibody against ochratoxin A that had been formed in rabbits after injection of an albumin-ochratoxin A conjugate (Elling, 1977).

Groups of 80 F 344/N rats of each sex were administered 0, 21, 70, or 210 μg ochratoxin A/kg in corn oil by gavage, 5 days per week for 103 weeks (Boorman, 1988). The administration of ochratoxin A to male and female rats caused a spectrum of degenerative and proliferative changes in the kidney. The predominant non-neoplastic lesion in treated rats was degeneration of the renal tubular epithelium in the inner cortex and the outer stripe of the outer medulla (nephropathy).

[a] Tm_{PAH} = transport maximum for para-aminohippuric acid.
Cin = clearance of insulin.

In four pigs given 0.8 mg ochratoxin A/kg body weight orally for 5 consecutive days, the activity of catalase and CN-insensitive palmitoyl-CoA-dependent NAD (nicotinamide adenine dinucleotide) in renal homogenates decreased, but that in hepatic homogenates did not, suggesting peroxisomal changes. This was confirmed by ultrastructural observations of peroxisomes in the proximal tubules in kidneys of ochratoxin A-treated animals (Elling et al., 1985).

When 11 SPF pigs and 23 beagle dogs were given high oral doses of ochratoxin A corresponding to feed levels of more than 5–10 mg/kg (levels rarely found in nature), pathological effects, mainly necrosis, were observed in the liver, intestine, spleen, lymphoid tissue, leukocytes, and kidney (Szczech et al., 1973a,b,c). Three groups of Wistar rats, each consisting of 15 animals, were exposed to feed levels of ochratoxin A ranging from 0.2 to 5 mg/kg for 3 months. Renal damage in the form of tubular degeneration was observed at all dose levels (Munro et al., 1974). A decrease in urinary osmolality, glycosuria, and proteinuria were observed in an unstated number of Sprague-Dawley and Wistar rats administered daily doses intraperitoneally of 0.75–2 mg ochratoxin A/kg body weight (Berndt & Hayes, 1979).

In a study of coagulation factors, rats were given daily oral doses of 4 mg/kg body weight over 4–10 days, resulting in decreases in plasma-fibrinogen levels, at levels of factors II, VII, and X, and in the platelet and megakaryocyte counts (Galtier et al., 1979).

In 4 calves fed rations containing 0.1–2 mg ochratoxin A/kg body weight for 30 days, the only signs observed were polyuria, increased levels of glutamic oxalacetic transaminase (GOT) in serum, and mild enteritis (Pier et al., 1976); there was mild tubular degeneration in the kidneys. Cows given ochratoxin A orally for 4 days at doses ranging from 0.2 to 1.66 mg/kg body weight remained clinically normal (Ribelin et al., 1978). A cow given a single dose of 13.3 mg/kg body weight (corresponding to 865 mg/kg feed) developed diarrhoea, anorexia, and cessation of milk production, one day after.

Avian nephropathy, similar to that in spontaneously occurring cases, developed in Leghorn hens (27 birds per group) exposed to dietary levels of 0.3 or 1 mg ochratoxin A/kg for one year

(Krogh et al., 1976c). The renal changes included degeneration of the tubular epithelium, mainly confined to the proximal and distal tubules of both reptilian and mammalian nephrons; impairment of glomerular and tubular function was also observed. "Acute nephrosis" and "visceral gout" were observed in chickens exposed to high levels of ochratoxin A (LD_{50} values) (Peckham et al., 1971). The same authors reported that ochratoxin B, the other naturally occurring ochratoxin, was not highly toxic for chickens (LD_{50}, 54 mg/kg) or other animals.

Groups of chicks (40 birds per group) were fed diets containing ochratoxin A levels in the range of 0–8 mg/kg for 3 weeks (Huff et al., 1974). The group receiving the highest dose (8 mg/kg feed) showed decreased packed blood cell volume, haemoglobin concentration, and serum-iron and serum-transferrin saturation percentages. In a similar study, Chang et al.(1979) observed that lymphocytopenia developed at all dose levels. In the same study, decreased bone strength, as measured physically by resistance to fracturation, was observed at feed levels of 2–4 mg ochratoxin A/kg (Huff et al., 1980). When groups of chicks were fed ochratoxin A at levels of 0, 2, or 4 mg/kg for 20 days, concentrations of serum-immunoglobulins (IgA, IgG, IgM) were reduced to 57–66% of normal values in the toxin-exposed groups (Dwivedi & Burns, 1984).

In B6C3F1 female mice, groups of 6–7 animals were administered a total of 0, 20, 40, or 80 mg ochratoxin A/kg body weight ip on alternate days over an 8-day period. A dose-related decrease in thymic mass was observed as well as myelotoxicity, indicated by bone marrow hypocellularity, due to decreased marrow pluripotent stem cells, and granulocyte-macrophage progenitors (Boorman et al., 1984).

When determining the LD_{50} in mice following intraperitoneal injection, synergistic effects were observed when ochratoxin A was combined with citrinin as well as with penicillic acid (Sansing et al., 1976). An additive effect was observed between ochratoxin A and citrinin in terms of embryotoxicity in chicken embryos (Vesela et al., 1983). In beagle dogs, when combined oral doses of ochratoxin A (0.1–0.2 mg/kg body weight) and citrinin (5–10 mg/kg) were injected intraperitoneally, synergism was

observed with regard to severity of clinical disease and mortality (Kitchen et al., 1977a,b). Increased toxicity in terms of LD_{50} values and pathological changes was observed in rats, when the ochratoxin A was given orally combined with either of the drugs biscoumacetate or phenylbutazone, apparently because of displacement of the toxin from binding sites on plasma-proteins (Galtier et al., 1980). The toxic effects of ochratoxin A on the renal epithelial cells of the monkey were demonstrated in *in vitro* studies in the form of abnormal mitotic cells (Steyn et al., 1975).

I.4.2.2 Teratogenicity

Intraperitoneal injection of pregnant mice with ochratoxin A at 5 mg/kg body weight on one of gestation days 7–12 resulted in increased prenatal mortality, decreased fetal weight, and various fetal malformations, including exencephaly and anomalies of the eyes, face, digits, and tail (Hayes et al., 1974). When a combination of ochratoxin A (2 or 4 mg/kg body weight) and T-2 toxin (0.5 mg/kg body weight) was injected intraperitoneally in mice on gestation day 8 or 10, ochratoxin A exacerbated the incidence of T-2-induced gross malformations (tail and limb anomalies); increased fetocidal effects were also noted (Hood et al., 1978). Mice were given 3–5 mg ochratoxin A/kg body weight intraperitoneally or orally on gestation days 8, 9, and 10 or 15, 16, and 17 (Szczech & Hood, 1981). Cerebral necrosis was found in most fetuses from dams treated on days 15–17, but no cerebral necrosis developed after treatment on days 8–10, when ochratoxin A is overtly teratogenic. Pups of mice given 1.25 and 2.25 mg ochratoxin A/kg orally on gestation days 15, 16, and 17 were tested for surface righting, swimming, and pivoting (Poppe et al., 1983). The results of all 3 tests indicated that a delay in development had occurred; no dose-related pathological alterations were found.

Pregnant mice were administered 1–2 mg ochratoxin A/kg body weight, and/or 5–20 mg zearalenone/kg body weight or 0.125 mg diethylstilboestrol/kg body weight, orally, on day 9 of pregnancy, either individually or in combination, and the offspring were examined on day 19 (Arora et al., 1983). Teratogenic effects produced by ochratoxin A, such as exencephaly, open eyelids,

and microphthalmia, were reduced or absent when the toxin was given in combination with one of the 2 non-steroidal estrogenic substances, zearalenone or diethylstilboestrol.

Rats were treated orally with ochratoxin A at 0.25, 0.50, 0.75, 1, 2, 4, or 8 mg/kg body weight on gestation days 6–15 (Brown et al., 1976). Maternal toxicity was not observed below 4 mg/kg body weight, but an increased incidence of fetal resorptions was observed from 0.75 mg/kg body weight. All fetuses from dams given 0.25–0.75 mg/kg body weight weighed significantly less than control fetuses, and fetuses from dams given 0.75 or 1 mg/kg body weight were stunted. Reduced litters and decreased fetal weight were observed in rats administered 5 mg ochratoxin A/kg body weight, orally, on gestation day 8 (Moré et al., 1978).

Subcutaneous administration of ochratoxin A to rats (1.75 mg/kg body weight) on gestation days 5–7 resulted in the highest number of malformations, including hydrocephaly, omphalocele, and anophthalmia as well as a shift in position of the oesophagus (Mayura et al., 1982); lower doses (0.5 and 1 mg ochratoxin A/kg body weight) did not have any teratogenic effects, and higher doses (5 mg/kg) caused all fetuses to be resorbed. In a subsequent study by the same authors (Mayura et al., 1983), using the same ochratoxin A exposure conditions, it was shown that a diet low in protein (10% of protein concentration in normal rat feed) enhanced the teratogenic action of ochratoxin A in the rat. The combined action of ochratoxin A exposure and a low protein diet also resulted in decreases in mating and fertilization rates (22% and 39%, respectively) compared with the control group.

When rats were exposed to ochratoxin A and citrinin (another nephrotoxic mycotoxin produced by species of the *Aspergillus* and *Penicillium* genera) either singly or combined, enhanced teratogenic effects were observed in terms of gross malformations, visceral anomalies, and skeletal defects following combined oral administration of 1 mg ochratoxin A/kg body weight and 30 mg citrinin/kg body weight (Mayura et al., 1984). Maternal deaths (22–40%) occurred after the administration of the combined dose on days 5, 6, 7, and 14 of gestation, whereas administration of individual toxins did not cause any maternal

deaths and only minimal malformations no matter which gestation day they were administered.

Increased prenatal mortality and malformations, including hydrocephaly, micrognathia, and heart defects, were observed in hamsters injected intraperitoneally with ochratoxin A at doses of 5–20 mg/kg body weight on one of gestation days 7–9 (Hood et al., 1976).

I.4.2.3 Mutagenicity

Ochratoxin A did not have any effects in a *Bacillus subtilis* Rec-assay, measuring DNA damage when tested at 20 and 100 μg/plate (Ueno & Kubota, 1976). Ochratoxin A was not mutagenic to *Salmonella typhimurium* TA 1535, TA 1537, TA 1538, TA 98, or TA 100 at doses of up to 500 μg/plate, with or without exogenous metabolic activation (Kuczuk et al., 1978; Wehner et al., 1978b). No increase was observed in genetic changes at the ade 2 locus of *Saccharomyces cerevisiae* after treatment with 50 or 100 μg/plate ochratoxin A, with or without exogenous metabolic activation (Kuczuk et al., 1978). Ochratoxin A did not induce mutations to 8-azaguanine resistance in C3H mouse mammary carcinoma cells (FM3A) treated with doses of 5 or 10 μg/litre (Umeda et al., 1977).

When rats were orally exposed to ochratoxin A every 48 h for 12 weeks (corresponding to a feed level of 4 mg/kg), single-strand breaks of DNA in renal and hepatic tissue (the only tissues investigated) were more pronounced than those in control animals (Kane et al., 1986a,b).

Contradictory results have been obtained by testing ochratoxin A in the *Salmonella* assay (SOS chromo test without metabolic activation) (Ueno et al., in press).

I.4.2.4 Carcinogenicity

Kanizawa & Suzuki (1978) have indicated that ochratoxin A is a hepatic and renal carcinogen in male mice. A group of 10 male ddY mice were fed a diet containing 40 mg ochratoxin A/kg for 44 weeks. A group of 10 untreated controls were fed the basal

diet. All survivors were killed after 49 weeks. Hepatic cell tumours were found in 5 out of 9 treated mice; no tumours were found in the 10 controls. Solid renal cell tumours were found in 2 out of 9 treated mice and none in the 10 controls. Cystic renal adenomas were found in 9 out of 10 treated mice compared with none in the 10 controls.

Two groups of 50 male and 2 groups of 50 female B6C3F1 mice were fed diets containing 1 or 40 mg ochratoxin A/kg, respectively; one group (control) was fed the basal diet. All survivors were killed after 24 months. Eleven out of 49 male mice in the 40 mg/kg group had renal carcinomas; 24 out of 49 male mice in the 40 mg/kg groups showed renal adenomas. All male mice in the 40 mg/kg group had microscopic evidence of nephropathy. A few females in the 40 mg/kg group exhibited nephropathic changes but no carcinomas or adenomas. Compound-related lesions were absent in the controls and the 1 mg/kg groups (Bendele et al., 1985). In a test for the development of hyperplastic liver nodules in rats, ochratoxin A was characterized as having both an initiating and a promoting activity (Imaida et al., 1982).

Seven groups of 16 male ddY mice each were fed a diet containing 50 mg ochratoxin A/kg for various periods ranging from 0 to 30 weeks followed by feeding of the basal diet until the end of 70 weeks (Kanizawa, 1984). After 15 weeks of ochratoxin A exposure, 3 out of 15 animals had renal cell tumours; after 20 weeks, 1 out of 14 mice had renal cell tumours and 2 had hepatomas; after 25 weeks of exposure, 2 out of 15 mice had renal cell tumours and 5 had hepatomas; and after 30 weeks of exposure, 4 out of 17 mice had renal cell tumours and 6 had hepatomas; these tumours were not observed in the controls. The nature of the renal cell tumours was not further defined, but such tumours are mostly malignant. In addition, pulmonary tumours were found in all groups, including the controls, the incidence ranging from 20 to 73%. In a second study, the effects of a combination of ochratoxin A and citrinin were elucidated. Groups of 20 male ddY mice were fed diets containing 25 mg ochratoxin A/kg in combination with 100 or 200 mg citrinin/kg for 70 weeks. Control groups were fed diets that did not contain any toxins or the individual toxins at the concentrations indicated above. In

addition, one group was fed 25 mg ochratoxin A/kg feed for the first 25 weeks followed by 200 mg citrinin/kg feed for the remaining period of time; another group was exposed to the 2 toxins in the reverse order. Exposure to citrinin alone did not produce any tumours. Exposure to ochratoxin A alone resulted in renal cell tumours in 6 out of 20 mice, and in hepatomas in 8 mice. Exposure to ochratoxin A and 100 mg citrinin/kg feed did not result in any renal cell tumours, but 10 out of 19 mice had hepatomas. Exposure to ochratoxin A and 200 mg citrinin/kg feed resulted in renal cell tumours in 10 out of 18 mice, and in hepatomas in 7 mice. Exposure to one toxin followed by the other toxin did not produce any renal cell tumours, but hepatomas were observed in less than 20% of the mice.

On the basis of these studies, IARC concluded that there was limited evidence of carcinogenicity for animals, and inadequate evidence of carcinogenicity for human beings (IARC, 1987a).

Groups of 80 F 344/N rats of each sex were administered 0, 21, 70 or 210 μg ochratoxin A/kg in corn oil by gavage, 5 days per week, for 103 weeks (Boorman, 1988). In the male rats, renal carcinomas were found in 16 out of 51 animals dosed with 70 μg/kg and in 30 out of 50 animals dosed with 210 μg/kg; no carcinomas were found in lower dose groups. In the female rats, renal carcinomas were less common, as 1 out of 50 animals dosed with 70 μg/kg and 3 out of 50 animals dosed with 210 μg/kg had carcinomas; no carcinomas were found in the lower dose groups. Renal adenomas were found in all groups of male rats, with increasing frequencies associated with increased doses. In the female groups, renal adenomas were only found in the two highest dose groups. In the female rats, fibroadenomas in the mammary gland were found in 45–56% in the treated groups, a significantly higher percentage than in the control group.

I.4.2.5 Biochemical effects and mode of action

Ochratoxin A is an inhibitor of tRNA synthetase and protein synthesis in several microorganisms (*Bacillus subtilis, B. stearothermophilus, Streptococcus faecalis*, yeasts) as well as in rat hepatoma cells (Konrad & Roschenthaler, 1977; Bunge et al., 1978; Heller & Roschenthaler, 1978; Creppy et al., 1979a,b). The

competitive inhibitor effect of ochratoxin A on tRNA synthetase and protein synthesis in rat hepatoma cells can be prevented by the addition of phenylalanine in the cell culture medium at a molar ratio of phenylalanine: ochratoxin A of 1.7:1 (Creppy et al., 1979b). This observation suggested the possibility of preventive measures for ochratoxin A-induced disease. Thus, the acute intraperitoneal effect of ochratoxin A (LD_{100}) in mice was prevented by concomitant injection of phenylalanine (Creppy et al., 1980; Moroi et al., 1985).

However, in the study on the reproduction of porcine nethropathy previously mentioned (Krogh et al., 1974a), the molar ratio of phenylalanine: ochratoxin A in the feed exceeded 4600:1, implying that, in the field situation, phenylalanine does not prevent ochratoxin A from inducing the development of nephropathy.

Ochratoxin A in the concentration range studied (20–1667 μmol/litre) caused a 47–50% inhibition of macrophage migration (Klinkert et al., 1981); this effect could be prevented by the simultaneous addition of phenylalanine. In BALB/c mice, a dose of ochratoxin A as low as 0.005 μg/kg body weight was able to suppress the immune response to sheep erythrocytes (Haubeck et al., 1981); the effect could be prevented by the simultaneous addition of phenylalanine. Studying the same effect in Swiss mice, Prior & Sisodia (1982) were unable to show any suppression of the immune response to sheep erythrocytes, even after daily injection of 5 mg ochratoxin A/kg for 50 days. (4R)-4-Hydroxyochratoxin A at a dose of 1 μg/kg body weight caused an 80% reduction in the number of cells producing IgM and a 93% reduction in cells producing IgG in BALB/c mice compared with 90% and 92%, respectively, for ochratoxin A (Creppy et al., 1983).

Female B6C3F1 mice (6 per group) were administered ochratoxin A in amounts of 0.34, 6.7, or 13.4 mg/kg body weight or ochratoxin B (13.4 mg/kg body weight) 6 times during 12 days. Ochratoxin A inhibited the natural killer cell activity at all dose levels, and increased the growth of transplantable tumour cells without affecting T-cell or macrophage-mediated antitumour activity (Luster et al., 1987). Ochratoxin B did not influence immune function. The inhibition by ochratoxin A of

natural killer cell activity appeared to be caused by reduced production of basal interferon.

Ochratoxin A affects the carbohydrate metabolism in rats. Thus, a single oral dose of ochratoxin A at 15 mg/kg body weight caused a decrease in the glycogen level in the liver and an increase in the heart glycogen level, 4 h later (Suzuki & Satoh, 1973).

In a more extensive study on rats, the decrease in liver glycogen level, 4 h after a single oral dose of ochratoxin A at 15 mg/kg body weight, was associated with an increase in serum glucose levels and a decrease in liver glucose-6-phosphate (Suzuki et al., 1975). At the same time, the liver glycogen synthetase (EC 2.7.1.37) activity decreased and the liver phosphorylase (EC 2.4.1.1) activity increased. Three daily oral doses of ochratoxin A at 5 mg/kg body weight caused a decrease in the liver glycogen concentration, which was measured on the fourth day. The decrease was attributed to inhibition of the active transport of glucose into the liver, suppression of glycogen synthesis from glucose, and acceleration of glycogen decomposition.

During *in vitro* studies on rat liver mitochondria, it was observed that ochratoxin A inhibited the respiration of whole mitochondria by acting as a competitive inhibitor of transport carrier proteins located in the inner mitochondrial membrane (Meisner & Chan, 1974). Further studies with mitochondrial preparations revealed that the mitochondrial uptake of ochratoxin A was an energy-using process that resulted in depletion of intramitochondrial adenosine triphosphate (ATP), and that ochratoxin A inhibited intramitochondrial phosphate transport resulting in deterioration of the mitochondria (Meisner, 1976). This might explain the degeneration of liver mitochondria observed by Purchase & Theron (1968) in rats exposed orally to a single dose of ochratoxin A at 10 mg/kg body weight. These authors observed accumulation of glycogen in the cytoplasm of the rat liver cells microscopically. This was in contrast to the previously discussed observations of Suzuki et al. (1975), who found a decrease in glycogen levels.

In a study on mice, Sansing et al. (1976) found that ochratoxin A, administered intraperitoneally at 6 mg/kg body weight, inhibited orotic acid incorporation into both liver and kidney RNA,

6 h after toxin injection. In this respect, ochratoxin A acted synergistically with another nephrotoxic mycotoxin, citrinin.

When neonatal rats were exposed orally to a single dose of 1 mg ochratoxin A/kg or of 25 mg citrinin/kg or both doses within 24 h of birth, a synergistic effect of the 2 mycotoxins was observed on cytochrome P-450, NADPH-dependent dehydrogenase, and NADPH-cytochrome *c* reductase (Siraj et al., 1981).

In rats fed 2 mg ochratoxin A/kg body weight per day for 2 days, renal gluconeogenesis from pyruvate was decreased by 26%, and renal phosphoenol-pyruvate carboxy kinase (PEPCK) (EC 4.1.1.32) activity was reduced by 55%, whereas hepatic PEPCK was unchanged (Meisner & Selanik, 1979). In a subsequent study on rats, it was found that even lower dose levels (0.3–0.5 mg ochratoxin A/kg body weight) caused a 50% reduction in PEPCK activity (Meisner & Meisner, 1981). A number of other enzymes located in the proximal tubule of the nephron were unaffected. When longer exposure periods (8–12 weeks) were used, and 145 ng ochratoxin A/kg body weight were administered orally to groups of 3 rats each, increased urinary excretion and corresponding renal tubular depletion of the following enzymes were observed: gamma-glutamyl transferase, alkaline phosphatase, leucine aminopeptidase, lactate dehydrogenase, and *N*-acetyl-beta-*D*-glucosaminidase (Kane et al., 1986a). Renal PEPCK in pigs was also sensitive to ochratoxin A with a feed level of 100 μg/kg causing a significant decrease in PEPCK activity (Meisner & Krogh, 1982).

In a study on pigs fed ochratoxin A at 0, 0.2, or 1 mg/kg for 5 weeks, enzyme activities were measured in renal biopsies, collected 1, 3, and 5 weeks after initiation (Krogh et al., 1988). After one week, the activities of renal PEPCK and gamma-glutamyl-transpeptidase were decreased by 40%. The dose-related decrease in the activity of PEPCK and gamma-glutamyl-transpeptidase was accompanied by a dose-related increase in renal impairment, as measured by the reduction of Tm_{PAH}/Cin, suggesting that these enzymes are sensitive indicators of ochratoxin-induced porcine nephropathy. Thus, the renal biopsy-based measurements of enzyme activities might prove

diagnostically useful in ochratoxin-induced disease in human beings.

Ochratoxin A reduced the total renal mRNA concentration in male Sprague-Dawley rats and certain mRNA species, notably PEPCK, were reduced to a greater extent than the bulk of the RNA pool (Meisner et al., 1983).

I.5 EFFECTS ON MAN

I.5.1 Ochratoxin A, Balkan endemic nephropathy, and tumours of the urinary system

This topic has been reviewed by Krogh (1979, 1983) who called attention to the striking similarities in the changes in renal structure and function induced experimentally in animals by the administration of ochratoxin A, and the clinical and pathological features of a localized endemic disease known as Balkan endemic nephropathy. So far, the disease has been observed only in rural populations of Bulgaria, Romania, and Yugoslavia, but information on the present magnitude of the problem was not available to the Task Group.

The Balkan endemic nephropathy is a chronic disease that predominantly affects women and progresses slowly up to death (Hrabar et al., 1976, Chernozemsky et al. 1977). Age-specific incidence rates are highest above the age of 40. Younger cases occur in the 10–19-year-old age group, and the mean age of new patients is in the early 50s (Stoyanov et al., 1978).

The disease is characterized by an extreme geographical clustering with a tendency for familial aggregation of cases (Nicolov et al. 1978). However, sporadic cases occur outside endemic areas. Age-adjusted incidence rates of 555 per 100 000 population in females and 322 in males over a ten-year period have been recorded from a population sample of 147 000 in an endemic area of Bulgaria (Stoyanov et al. 1978). In one of several endemic regions in Yugoslavia, the prevalence varied from 3% to 8% (Hrabar et al., 1976).

Autopsy has shown that kidneys are notably reduced in size. The histological lesions are interstitial fibrosis, tubular degeneration, and hyalization of glomeruli in the more superficial part of the cortex (Heptinstall 1974). Impairment of tubular function, indicated by a decrease in Tm_{PAH}, is a prominent and early sign (Dotchev 1973).

A high incidence of tumours of the urinary system is strongly correlated with the prevalence of Balkan endemic nephropathy (Ceovic et al., 1976; Chernozemsky et al. 1977; Nicolov et al., 1978). In one instance in Bulgaria, 46.6% of patients with tumours of the urinary system were also affected by endemic nephropathy. Among the tumours of the urinary system, cancers of the renal pelvis and ureters are more frequently associated with endemic nephropathy than urinary bladder tumours.

The relative risk for developing cancer of the renal pelvis and ureters is 88:1 in patients with nephropathy compared with controls in non-endemic areas. The relative risk for the development of any tumour of the urinary system is only 28:1 in the same sample (Stoyanov et al., 1978).

Over the past 2 decades, investigations have been carried out to verify a variety of etiological assumptions with unconvincing results (review by Puchlev, 1973, 1974). However, the assumption of the possible role of ochratoxin A, on the basis of similarities with the animal disease, has received increasing epidemiological support.

In Yugoslavia, surveys indicated that the contamination of foodstuffs (grains, maize, pork meat) with ochratoxin A occurred in 12.8% of samples in an area where the prevalence of endemic nephropathy was 7.3%, compared with only 1.6% of contaminated samples in areas free of the disease (Krogh et al., 1977). The concentration of ochratoxin A in maize was 5–90 μg/kg and that in pork meat, 5 μg/kg (Krogh et al. 1977) with levels of up to 27 μg/kg in pig kidneys (Pepeljnjak et al. 1982). Similarly, studies in Bulgaria have revealed that 16.7% of beans and 27.3% of maize from an endemic area were contaminated with ochratoxin A compared with 7.1% and 9%, respectively, from a non-endemic area (Petkova-Bocharova & Castegnaro 1985).

In a subsequent survey of home-produced foodstuffs (cereal and bread) from the same endemic area in Yugoslavia over a 5-year period, a mean contamination of 8.7% was found with pronounced annual variations, which probably reflect climatic conditions during the crop harvesting periods (Pavlovic et al., 1979).

Surveys on the presence of ochratoxin A in blood samples are difficult to compare, as different analytical methods have been used with different levels of sensitivity. Prevalences of 16.6% and 5.9% with a one-year interval were reported in the same endemic village in Yugoslavia (Hult et al. 1982). It is not possible to determine whether this difference reflects annual variations in the content of the blood, or the result of a less sensitive analytical method in the second instance. However, higher prevalences of ochratoxin A and higher concentrations in blood are generally present in people from endemic areas, especially in persons suffering from Balkan nephropathy.

A survey in Yugoslavia reported a prevalence in the blood of 16.6% in an endemic village and 6% in a non-affected one (Hult et al. 1982). In Bulgaria, reported rates were 17.7% in an endemic area and 7.7% in a non-endemic one (Petkova-Bocharova et al., 1988). Table 9 illustrates the trend towards higher concentrations in endemic situations and in patients. The similarity of results in healthy families from affected villages and healthy persons from unaffected villages (groups III and IV) suggests than an environmental or a behavioural determinant plays a role at the household level.

Thus, available epidemiological information seems to indicate that Balkan endemic nephropathy is associated with consumption patterns involving foodstuffs contaminated with ochratoxin A and with a higher frequency of positive blood samples of ochratoxin A. However, the association does not permit the establishment of a causal relationship. Cross sectional surveys, such as those reported in the literature so far, are probably not the appropriate means to determine this relationship.

The results of experimental animal studies suggest that Balkan nephropathy, a chronic condition, may require a long latency period between exposure and the onset of symptoms, or more likely a prolonged exposure or repeated exposure over a long period of time.

Investigations based on individual exposure time sequences together with follow-up cohort surveys could provide a clue to the causal role of ochratoxin A in Balkan endemic nephropathy.

In view of the lack of this information, such a causal relationship cannot be established or rejected.

Table 9. Ochratoxin A in blood samples from people in endemic and non-endemic areas in Bulgaria[a]

Group[b]	No. of persons assayed	No. of ochratoxin A positive cases (%)	Mean concentration ± S.D. (μg/kg)
I. Patients with UST and/or EN	61	16 (26.3)	20.3 ± 9.7
II. Healthy persons from families with UST and/or EN cases	63	10 (15.8)	14.5 ± 7.6
III. Healthy persons from families in endemic villages	63	7 (11.1)	12.5 ± 3.5
IV. Healthy persons from unaffected villages in endemic areas	60	7 (11.6)	15.0 ± 4.2
V. Healthy persons from villages in non-endemic areas	65	5 (7.7)	10.0

[a] From: Petkova-Bocharova et al., (1988).
[b] UST: urinary system tumours; EN: endemic nephropathy.
The differences between groups I and groups III and IV are statistically significant ($P < 0.002$). Difference between groups I and V is statistically significant ($P < 0.001$).

I.6 EVALUATION OF THE HUMAN HEALTH RISKS

Human exposure, as demonstrated by the occurrence of ochratoxin A in food and in the blood, has been observed in various countries in Europe. The Task Group was not aware of attempts to detect ochratoxin A in human blood in other parts of the world.

The causal role of ochratoxin A in porcine nephropathy has been established, based on studies of field cases as well as reproduction of the disease with ochratoxin A. Using the porcine model, it has been postulated that Balkan endemic nephropathy may result from exposure to ochratoxin A. Available epidemiological information indicates that Balkan nephropathy may be associated with the consumption of foodstuffs contaminated by this toxin. Since the publication of Environmental Health Criteria 11, in 1979, epidemiological studies on the concentration of ochratoxin A in human blood in affected and non-affected areas, have provided additional support for the relationship between Balkan nephropathy and exposure to ochratoxin A.

It has been shown that both the prevalence of ochratoxin A in the blood and the blood concentrations are higher in residents in endemic areas. However, a direct causal relationship cannot be established on the basis of indirect evidence provided by the above retrospective studies alone. Neither can it be excluded in view of the long latency period between the exposure and the onset of symptoms.

Ochratoxin A has been demonstrated to be carcinogenic to the renal tubular epithelium in male mice and both sexes of rats. A highly significant relationship has been observed between Balkan nephropathy and tumours of the urinary tract, particularly with tumours of the renal pelvis and ureters. However, there are no published data to establish a direct causal role of ochratoxin A in the etiology of such tumours.

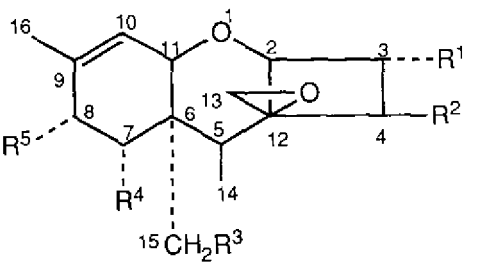
II. TRICHOTHECENES

II.1 PROPERTIES AND ANALYTICAL METHODS

II.1.1 Physical and chemical properties

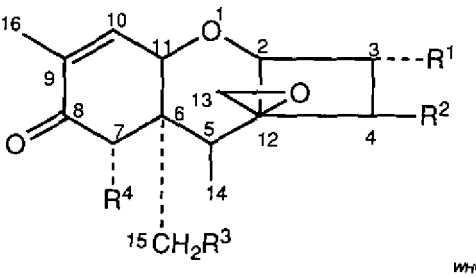
The sesquiterpenoid trichothecenes possess the tetracyclic 12,13-epoxytrichothecene skeleton. A total of 148 trichothecenes, 83 non-macrocyclic and 65 macrocyclic, have been isolated from fungal cultures and plants (Drove, 1988). They can be conveniently divided into 4 categories according to similarity of functional groups (Ueno, 1977). The first class is characterized by a functional group other than a ketone at C-8 (type A). This is the largest category containing members such as T-2 toxin and diacetoxyscirpenol (DAS). The second category of trichothecenes has a carbonyl function at C-8 (type B) typified by 4-deoxynivalenol (DON) and nivalenol (NIV). The third category is characterized by a second epoxide group at C-7,8 or C-9,10 (type C), and the fourth contains a macrocyclic ring system between C-4 and C-15 with two ester linkages (type D). The structures of representative trichothecenes of each category are illustrated below.

Type A Trichothecenes



Name	R_1	R_2	R_3	R_4	R_5
T-2 toxin	OH	OAc	OAc	H	$OCOCH_2CH(CH_3)_2$
T-2 tetraol	OH	OH	OH	H	OH
HT-2 toxin	OH	OH	OAc	H	$OCOCH_2CH(CH_3)_2$
Diacetoxyscirpenol	OH	OAc	OAc	H	H
Neosolaniol	OH	OAc	OAc	H	OH

Type B Trichothecenes

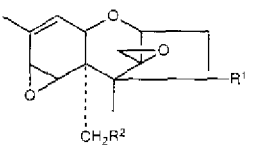


WHO 90528

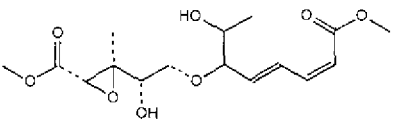
Name	R_1	R_2	R_3	R_4
Deoxynivalenol	OH	H	OH	OH
Nivalenol	OH	OH	OH	OH
Trichothecin	H	$OCOCH=CHCH_3$	H	H
Fusarenon-X	OH	OAc	OH	OH

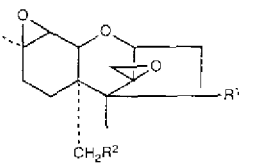
Type C Trichothecenes

Name	R_1	R_2
Crotocin	H	$-OCOCH=CHCH_3$

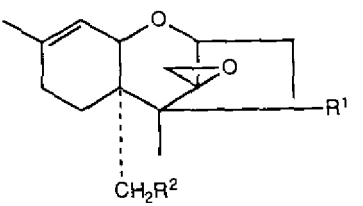


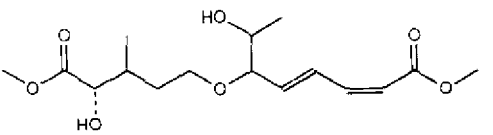
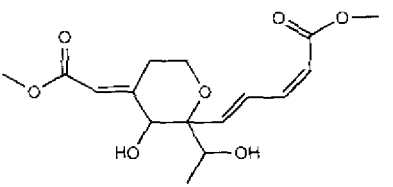
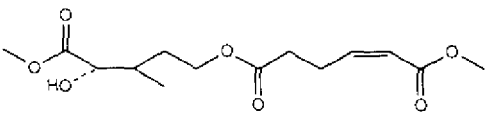
Baccharin





Type D Trichothecenes



Name	R_1	R_2
Roridin A		
Satratoxin H		
Verrucarin A		

II.1.1.1 Physical properties

The trichothecenes are colourless, mostly crystalline solids that have been well characterized by physical and spectroscopic techniques (Cole & Cox, 1981). The type A trichothecenes are soluble in moderately polar solvents, such as chloroform, diethyl ether, ethyl acetate, and acetone, whereas the more polar type B trichothecenes require higher polarity solvents, such as aqueous

methanol or aqueous acetonitrile. Some physical properties of the main trichothecenes are summarized in Table 10.

Most of the trichothecenes lack conjugated unsaturation in their structures with a consequent absence of absorption in the ultraviolet (UV) spectrum, except for end absorption due to unsaturation at C-9. This lack of absorbance is a source of difficulty in achieving sensitive and specific detection in HPLC analysis. In contrast, the type D trichothecenes give characteristic ultraviolet spectra.

II.1.1.2 Chemical properties

When trichothecenes containing an ester group are treated with a base, they are hydrolysed to their corresponding parent alcohol (Wei et al., 1971). Free hydroxyl groups are readily acylated. The 12,13-epoxy group is itself extremely stable to nucleophilic attack. However, prolonged boiling under highly acidic conditions causes an intramolecular rearrangement of the trichothecene skeleton to the apotrichothecene ring. Detailed discussion of the chemistry of trichothecenes can be found in reviews by Bamburg & Strong (1971), Bamburg (1976), and Tamm (1977).

The trichothecenes are generally stable; for example, DON can be stored in organic solvents, such as ethyl acetate, for a long time without any significant deterioration (Shepherd & Gilbert, 1988). They remain unaffected when refluxed with various organic solvents and also under mildly acidic conditions.

II.1.2 Analytical methods for trichothecenes

Analytical methods have been reviewed by Scott (1982) and Pohland et al. (1986). Selected examples of recently published analytical methods for type A trichothecenes and type B trichothecenes are summarized in Tables 11 and 12 respectively. Although some of the multi-trichothecene methods included in Table 11 may also be applicable to certain of the type B compounds, the procedures in Table 12 have been developed exclusively for the type B toxins.

Table 10. Some physical properties of main trichothecenes

Trichothecenes	Molecular formula	Relative molecular mass	Melting point (°C)	$(\alpha)_D^{20}$	References
T-2 toxin	$C_{24}H_{34}O_9$	466	151–152	+15	Bamburg et al. (1968b)
HT-2 toxin	$C_{22}H_{26}O_8$	424	-	-	Bamburg & Strong (1969)
Diacetoxyscirpenol	$C_{19}H_{26}O_7$	366	162–164	–27	Sigg et al. (1965)
Neosolaniol	$C_{19}H_{26}O_8$	382	171–172	-	Ishii et al. (1971)
Deoxynivalenol	$C_{15}H_{20}O_6$	296	151–153	+6.35	Yoshizawa & Morooka (1973)
Nivalenol	$C_{15}H_{20}O_7$	312	222–223	+21.54	Tatsuno et al. (1968)
Trichothecin	$C_{19}H_{24}O_5$	332	118	+44	Freeman (1955)
Fusarenon-X	$C_{17}H_{22}O_8$	354	91–92	+58	Ueno et a.l. (1969b)
Roridin A	$C_{29}H_{40}O_9$	532	198–204	+130	Harri et al. (1962)
Satratoxin H	$C_{29}H_{36}O_9$	528	162–166	-	Eppley & Bailey (1973)
Verrucarin A	$C_{27}H_{34}O_9$	502	360	+20.6	Gutzwiller & Tamm (1965)

Table 11. Selected methods for the determination of T-2 toxin and diacetoxyscirpenol in biological materials

Matrix	Extraction[a]	Clean-up[b]	Assay[c]	Detection limit (μg/g)	Toxins assayed			References
					T-2	DAS	Others	
Cereals	MeOH/H_2O	XAD-4; flor.	TLC	0.5	+	+	7	Kamimura et al.(1981)
Cereals	EtOAC	prep. TLC	HPTLC	0.2	+	-	2	Ilus et al.(1981)
Foods	MeCN/H_2O	char/alum	TLC	1.0	+	+	5	Romer (1986)
Cereals	MeCN/KCl	Sep P	HPLC (RI)	1.0	+	-	1	Schmidt & Dose (1984)
Wheat/rye	MeCN	Bond-Elut	LC/MS (therm)	0.04	+	+	2	Rajakyla et al.(1987)
Plasma	EtOAC	Sep P/sil.g	LC/MS (therm)	0.002	+	+	2	Voyksner et al.(1985)
Cereals	EtOAC	sil.g	GC (FID)-TMS	0.1	+	+	3	Bata et al. (1983)
Cereals	MeOH/H_2O	sil.g/cy	GC (ECD)-HFB	0.05	+	+	1	Cohen & Lapointe (1984)
Plasma	benzene	flor.	GC (ECD)-HFB	0.02	+	-	-	Swanson et al. (1983)
Milk	EtOAC	prep.TLC	GC/MS-TMS	0.003	+	-	-	Collins & Rosen (1979)
Corn	MeOH	Sep P/sil.g	GC/MS-TMS	0.02	+	+	1	Rosen & Rosen (1984)
Foods	MeOH	flor.	GC/MS-HFB	0.01	+	+	11	Black et al. (1987)
Urine	EtOAC/MeOH	Sep P	GC/MS-HFB	0.005	+	+	5	Black et al. (1986)
Blood	acetone	Sep P	GC/MS-PFP	0.005	+	+	9	Begley et al. (1986)
Milk/urine	EtOAC	Sep P	RIA	0.002	+	-	-	Lee & Chu (1981a)
Corn/wheat	MeOH	Sep P	RIA	0.007	+	-	-	Lee & Chu (1981b)
Urine	none	Sep P	ELISA	0.0005	+	-	-	Fan et al. (1987)

Table 11 (*continued*).

[a] Extraction :- MeOH (methanol); H_2O (water); EtOAc (ethyl acetate); MeCN (acetonitrile); KCl (potassium chloride aqueous soln).

[b] Clean-up :- XAD-4 (amberlite XAD-4 resin); flor. (florisil); char/alum (charcoal/alumina columns); Sep P (C_{18}-Sep Pak); sil.g (silica gel); cy (cyano extraction column).

[c] Assay :- TLC (thin layer chromatography); HPTLC (high performance TLC); HPLC (high performance liquid chromatography); RI (refractive index); LC/MS (combined HPLC/mass spectrometry); GC (gas chromatography); FID (flame ionisation detector); ECD (electron capture detector); TMS (trimethylsilyl derivative); HFB (hepafluorobutyryl ester derivative); PFP (pentafluoropropionyl ester); RIA (radioimmunoassay); ELISA (enzyme linked immunosorbent assay).

Table 12. Selected methods for the determination of deoxynivalenol and nivalenol in biological materials

Matrix	Extraction[a]	Clean-up[b]	Assay[c]	Detection limit (μg/g)	Toxins assayed			References
					T-2	DAS	Others	
Wheat/corn	MeCN/H_2O	char/alum	TLC	0.04–0.1	+	-	-	Trucksess et al.(1984)
Foods	MeCN/H_2O	char/alum; Sep P	TLC	0.5	+	-	-	Trucksess et al.(1986a)
Cereals	MeCN/H_2O/MeOH	char/alum/ppt	HPTLC	0.5	+	+	1	Trucksess et al.(1987)
Corn/rice	MeOH/H_2O/NaCl	liq/liq extr	HPLC (UV)	0.005	+	+	2	Visconti & Bottalico (1983a)
Corn/rice	MeOH/H_2O	none	HPLC (ED)	0.025	+	-	-	Sylvia et al.(1986)
Corn	MeOH/H_2O	prep. TLC	HPLC (UV)	0.01	+	-	-	Ehrlich et al.(1983)
Cereals	MeCN/H_2O	ion/char/alum	HPLC (UV)	0.05	+	+	-	Lauren & Greenhalgh (1987)
Cereals	MeOH	flor.	LC/MS (micro)	0.01	+	+	-	Tiebach et al.(1985)
Cereals	MeOH/H_2O	sil.g	GC(ECD)-TMS	0.02	+	+	-	Scott et al. (1986)
Cereals	MeCN/H_2O	flor/Sep P	GC(ECD)-TMS	0.002	+	+	-	Tanaka et al. (1986)
Wheat	chlor/EtOH	sil.g	GC(ECD)-HFB	0.1	+	-	-	Ware et al. (1986)
Cereals	chlor/EtOH	sil.g	GC(ECD)-HFB	0.02	+	-	-	Mulders & Impelen-Peek (1986)
Milk	EtOAc	Sep P	GC(ECD)-TMS	0.001	+	-	-	Swanson et al. (1986)
Corn/barley	MeOH/H_2O	sil.g	GC/MS - TMS	0.01	+	-	-	Gilbert et al. (1983a)
Foods	MeOH/H_2O	XAD-2/flor/Sep P	GC/MS - TMS	0.02	+	+	-	Yoshizawa & Hosokawa (1983)
Corn/wheat	Me CN/H_2O	none	ELISA	0.01	+	-	-	Xu et al. (1988)

Table 12 (*continued*).

[a] Extraction : MeCN (acetonitrile); H_2O (water); MeOH (methanol); NaCl (sodium chloride soln); chlor/EtOH (chloroform/ethanol); EtOAc (ethyl acetate)

[b] Clean-up : char/alum (charcoal/alumina column); Sep P (C_{18}-Sep Pak); ppt (lead acetate precipitation); liq/liq extr. (liquid/liquid extraction); ion (ion exchange resin); flor. (florisil); sil.g (silica gel); XAD-2 (Amberlite XAD-2 resin).

[c] Assay : TLC (thin layer chromatography); HPTLC (high performance TLC); HPLC (high performance liquid chromatography); UV (ultraviolet detection); ED (electrochemical detection); LC/MS (combined microbore-HPLC/mass spectrometry); GC (gas chromatography); ECD (electron capture detector); TMS (trimethylsilyl derivative); HFB (heptafluorobutyryl ester derivative); ELISA (enzyme linked immunosorbent assay).

II.1.2.1 Chemical methods

(*a*) *Extraction*

The most commonly used extraction solvents for trichothecenes are chloroform, ethyl acetate, methanol, acetonitrile, aqueous methanol, and aqueous acetonitrile. Chloroform, ethyl acetate, and acetonitrile have been successfully used for the extraction of T-2 toxin, DAS, and some of their partially hydrolysed derivatives in naturally contaminated cereals. Aqueous methanol and aqueous acetonitrile are the solvents of choice for the extraction of several trichothecenes of widely differing polarity as well as for the extraction of type B toxins alone. Methods differ according to the type of solvent used, whether samples are homogenised in a blender with the solvent or agitated with a wrist action shaker, and in the length of time of the extraction process. Spiking of samples with standards is not an adequate way of demonstrating the efficiency of extraction, and only methods validated with naturally contaminated material can be regarded as having been rigorously tested. Extraction procedures have been assessed for DON (Trenholm et al., 1985) and it has been demonstrated that longer extraction times are required for naturally contaminated samples than for those that have been spiked (at least 120 min shaking). It has also been shown that aqueous acetonitrile gives a cleaner extract than aqueous methanol.

(*b*) *Clean-up procedures*

The extent of sample clean-up required for a particular assay depends on the specificity of the detection procedure and the nature of the sample matrix. The less specific detection methods, such as TLC, require extensive sample clean-up while more sophisticated approaches, such as mass spectrometry and immunoassay, may only require minimal sample preparation.

Early methods that may have involved the use of conventional silica gel columns or preparative TLC for clean-up, have to a large extent been superseded by methods using prepacked cartridges, such as silica gel and C_{18}-Sep Paks, which are both more reliable and more convenient. Florisil columns are widely used for clean-up (e.g., see Tanaka et al., 1985a), and the one

step clean-up procedures using alumina/charcoal (Romer, 1986) or alumina/charcoal/Celite columns (Trucksess et al., 1984) have become widely adopted, particularly for DON and NIV assays.

(c) *Detection and quantification*

(i) *Thin-layer chromatography (TLC).* The lack of native fluorescence or UV absorbance of the trichothecenes means that TLC detection relies on the use of spray reagents for visualisation. Characteristic colours or fluorescence can be produced with sulfuric acid or *p*-anisaldehyde followed by heating at 110–120 °C (Scott et al., 1970; Ueno et al., 1973c). A general spray reagent for the 12,13-epoxy function is 4-(*p*-nitrobenzyl)pyridine, which produces a blue coloration on heating and treatment with a base (Takitani et al., 1979). A more sensitive, but somewhat elaborate, procedure, which again is specific for the epoxide function, involves reaction with nicotinamide and 2-acetylpyridine to produce fluorescent TLC spots (Sano et al., 1982). Diphenylindenone sulfonyl esters of trichothecenes can be formed prior to TLC and, subsequently, when sprayed with sodium methoxide can yield fluorescent spots at high sensitivity (Yagen et al., 1986).

Most of the above spray reagents, though frequently demonstrated as useful for the determination of standards or of relatively high concentrations of toxins in culture extracts, have not been well developed in conjunction with clean-up procedures for the determination of trichothecenes in naturally contaminated cereal samples or other foods. However, the exception has been the use of an aluminum chloride spray reagent for visualising the type B trichothecenes (Kamimura et al., 1981). On spraying the plates, heating for 10 min at 110 °C and then treating with a base, blue fluorescent spots are produced for DON, NIV, and fusarenon X. Using this approach, in conjunction with a clean-up utilising an alumina charcoal Celite column, a quantitative TLC procedure was developed using a fluorodensitometer for determining DON in wheat and corn (Trucksess et al. 1984); DON in processed grain products including breakfast cereals, corn syrup, and beer (Trucksess et al., 1986b); and for simultaneously monitoring DON, NIV, and fusarenon X in barley, corn, and wheat (Trucksess et al. 1987). This TLC procedure

was successfully collaboratively tested (Eppley et al., 1986) and was accepted as the AOAC first action method for DON in wheat.

(ii) *High performance liquid chromatography (HPLC).* HPLC has not proved particularly appropriate for the type A trichothecenes, which lack any significant UV absorption, making sensitive detection difficult. Refractive index detection has been employed, but at relatively high toxin levels (Schmidt & Dose, 1984). UV detection at short wavelength has been used for the determination of DON and NIV in cereals, and a number of methods have been reported that offer an advantage over alternative GC approaches in that they do not require derivatization of the toxins. A post-column derivatization procedure for DON and NIV has been developed (Sano et al., 1987) involving alkaline decomposition of the trichothecenes to generate formaldehyde and then reaction with methyl acetoacetate and ammonium acetate to form a fluorescent derivative. Although more sensitive and specific than UV detection, the approach does have the disadvantage of requiring rather elaborate instrumentation, in order to carry out the post column reaction. Electrochemical detection (Sylvia et al., 1986) looks particularly promising for the determination of DON by HPLC, offering both greater sensitivity and specificity than UV detection, and the possibility of analysis of sample extracts that have received minimal sample clean-up.

(iii) *Gas chromatography (GC).* A large number of GC methods have been published that differ in the approach to sample extraction, in sample clean-up, and in choice of derivative prior to GC analysis. Trichothecenes containing a hydroxyl group require derivatization, and the heptafluorobutyryl (HFB) ester and trimethylsilyl (TMS) ether derivatives have been most frequently used in conjunction with electron capture detection. Formation of the HFB derivatives is relatively time-consuming, but complete derivatization is easily achieved and the derivative once formed is stable for at least several days. However, poor reproducibility has been noted with HFB derivatives of DON (Mulders & Impelen-Peek, 1986) attributed to adsorption on to glass surfaces. The relatively high mass of the HFB derivatives can cause difficulties if GC/MS confirmation is required. In contrast, TMS derivatives are easily prepared, and are of suitable

mass for GC/MS, but, for some type B toxins, they may require optimization of conditions for complete derivatization (Gilbert et al., 1985). Scott & Kanhere (1986) have compiled retention data for 10 different trichothecenes as both TMS and HFB derivatives on capillary columns of different stationary phases. Trifluoroacetyl derivatives of trichothecenes are preferred by some workers (Kientz & Verweij, 1986), and pentafluoropropionyl esters have been used, particularly where detection has been by MS (Begley et al., 1986; Krishnamurthy & Sarver, 1986; Rood et al., 1988a).

An area where improvement in quantification of trichothecenes is still needed is in the selection of adequate internal standards. Compounds, such as methoxychlor (Romer et al., 1978), hexachlorobiphenyl (Blass et al., 1984), and alkanes (Ilus et al., 1981), have been used, but these differ significantly in structure from the trichothecenes. The deuterated TMS derivative of T-2 toxin has been used as an internal standard in GC/MS analysis (Rosen & Rosen, 1984), an isomer of T-2 toxin has been chemically synthesized (Stahr et al., 1981) as have 4-deoxyverrucarol and 16-hydroxyverrucarol for use as internal standards (Krishnamurthy et al., 1986).

Despite the many difficulties, a GC method using the HFB derivative has been successfully collaboratively tested (Ware et al., 1986) and subsequently adopted as an AOAC official first action method.

Other related trichothecenes have been determined in foods by GC methods, for example, the de-epoxidised metabolite of DON called DOM-1 has been determined in milk as both its TMS and HFB derivative (Swanson et al., 1986). Also, an isomer of DON was detected by capillary GC, as its TMS derivative in bread and breakfast cereal products prepared from flour naturally contaminated with DON (Greenhalgh et al., 1984).

(iv) *Mass spectrometry (MS).* Mass spectrometry has been used for the structural characterization of novel trichothecenes; for identification and confirmation of trichothecenes in biological materials, and as a sensitive and specific means of detection with GC, LC, or supercritical fluid chromatography (SFC) sample introduction.

The usefulness of negative ion chemical ionization (NICI) mass spectrometry with hydroxide ion reagent gas has been demonstrated in relation to producing relative molecular mass information, as well as fragment ions indicative of structure for type A and B trichothecenes (Brumley et al., 1982). The NICI technique has been applied to confirmation of the presence of DON in cereals and snack foods by rapid capillary GC introduction of the underivatized extract obtaining full scan spectra (Brumley et al., 1985), and by selected ion monitoring (Miles & Gurprasad, 1985).

NICI has also been used for selected ion monitoring GC/MS of the HFB derivatives of 13 different trichothecenes of both type A and type B in extracts from small samples of biological material (Krishnamurthy et al., 1986). GC/MS is the preferred approach using SIM in electron ionization (D'Agostino et al., 1986) or NICI (Begley et al., 1986) modes for biological samples where only small sample sizes are available but high sensitivity is required. For more routine surveys of cereal and food samples, particularly for DON and NIV, initial screening has normally been carried out using GC with an ECD and only selected positive samples have been confirmed by GC/MS (Tanaka et al., 1985a; Cohen & Lapointe, 1982).

Attempts have been made to determine the macrocyclic trichothecenes as TMS derivatives on short fused silica GC columns using GC/MS (Rosen et al., 1986). The approach has also been adopted of alkaline hydrolysis of the ring system of the macrocyclic compounds to yield verrucarols, which can then be determined as HFB derivatives by NICI GC/MS (Krishnamurthy et al., 1987). A similar approach using alkaline hydrolysis has been proposed (Rood et al., 1988a,b) as a basis for a general method for the determination of trichothecenes and metabolites by conversion to their corresponding parent alcohols prior to derivatization and GC or GC/MS determination.

Although LC/MS is really a research method, thermospray LC/MS is becoming more widely available and shows promise for the identification of low levels of trichothecenes in biological materials (Voyksner et al., 1985; Rajakyla et al., 1987). Another promising research method for the determination of both the

simple and the macrocyclic trichothecenes is supercritical fluid chromatography (SFC) combined with MS (Smith et al., 1985). Supercritical fluids can also be used for the on-line extraction from biological materials and direct introduction into the MS to monitor a number of different trichothecenes (Kalinoski et al., 1986).

II.1.2.2 Immunological methods

Chu et al. (1979) developed a radioimmunoassay (RIA) for T-2 toxin. The antibody preparation was obtained by immunizing rabbits with bovine serum albumin T-2 toxin hemisuccinate conjugate. The antibody had the greatest binding efficiency for T-2 toxin and less efficiency for HT-2 toxin. Cross reaction with other trichothecenes was either very slight or absent, and the limit of detection of the assay ranged from 1 to 20 ng.

This RIA was applied to the determination of T-2 toxin in agricultural commodities, biological fluids, and animal organs (Lee & Chu, 1981a,b; Hewetson et al., 1987). Xu et al. (1988) also reported an immunoassay using an antibody against triacetylated DON, for the determination of DON in wheat and corn with a limit of detection of about 0.02 μg/g.

A polyclonal enzyme-linked immunosorbent assay (ELISA) was developed for the rapid quantification of T-2 toxin in food and animal feed (Pestka et al., 1981). This assay, which could be undertaken in 2 h, had a limit of detection of 2.5 pg/assay and was used for the detection of T-2 toxin in *Fusarium*-infected corn (Gendloff et al., 1984). More recently monoclonal ELISAs have been developed for T-2 toxin and DON (Feuerstein et al., 1985; Hunter et al., 1985; Chiba et al., 1988). Although these monoclonal assays are of invariable specificity and the antibodies are available in unlimited supply, competitive indirect ELISAs using monoclonals are generally less sensitive than polyclonal-based ELISAs. However, Chiba et al. (1988) developed a sensitive ELISA for the detection of T-2 toxin with monoclonal antibodies at a limit of 2.5 pg/assay. This assay has been applied to the detection of T-2 toxin in wheat flour (Chiba et al., 1988) and in fungal cultures (Nagayama et al.,1988).

Production of useful antibodies against DON has proved more difficult than for other trichothecenes. Xu et al. (1988) adopted the approach of raising antibodies against triacetyl-DON, which requires acetylation of DON in an extract of the contaminated cereal prior to carrying out a direct ELISA. This assay has the required sensitivity but also the disadvantage of determining the total concentration of DON plus any other acetylated derivatives that may be present in the extract. A different approach to raising monoclonal antibodies to DON has been carried out by forming the hemisuccinyl derivative after protection of two of the available adjacent hydroxyls with a cyclic boronate ester (Casale et al., 1988). Some cross-reactivity to other trichothecenes and their de-epoxy metabolites was observed, but the assay does show considerable potential as a simple and rapid screening method for contaminated cereals and for detection in biological samples.

II.1.2.3 Biological methods

Biological methods are essential for working with field cases where the cause of an incident is unclear and evidence is sought to associate an observed biological effect with the presence of fungally contaminated material. Biological methods are also important as monitoring procedures in the isolation and purification of new or previously unrecognized toxins, prior to characterization by spectroscopic and chemical methods.

The classical and commonly used bioassay for trichothecenes is the skin necrotization test. Extracts prepared from samples (see section II.1.2.1) are applied in a single dose to the shaved back of a rabbit, rat, or guinea-pig. Toxic preparations applied in this way produce erythema, oedema, intradermal haemorrhage, and necrosis. The guinea-pig is the most sensitive of these animals to trichothecenes (Ueno et al., 1970). As little as 0.2 μg of T-2 toxin and DAS were detected on the skin of the rabbit and guinea-pig (Chung et al., 1974; Balzer et al., 1977). Only 10 g of the corn sample are needed for testing with an extraction and clean-up procedure recommended by Eppley (1968). Macrocyclic trichothecenes are highly irritant to the skin, but NIV and DON, which have a low skin-necrotizing potency, could be routinely missed in this assay (Ueno et al., 1970).

Brine shrimp (*Artemia salina* L.) larvae also provide a useful biological system for monitoring trichothecenes, such as T-2 toxin, DAS, and the macrocyclic trichothecenes. The limit of detection of this test for these toxins is approximately 0.04–0.4 mg/litre (Eppley, 1974).

The rabbit reticulocyte assay, based on the inhibition of uptake of ^{14}C-leucine in eukaryote cells, is highly specific for trichothecenes; ID_{50} (50% inhibitory dose) values are 0.03 mg/litre for T-2 toxin and DAS, and 2.0–3.0 mg/litre for DON and NIV (Ueno et al., 1969a, 1973c). By combining chemical methods with this assay, DON was successfully isolated from *Fusarium*-infected corn (Ishii et al., 1975).

II.2 SOURCES AND OCCURRENCE

II.2.1 Taxonomic considerations

The vast majority of trichothecenes are produced by the *Fusarium* species. Production of these metabolites depends, of course, on many factors, including substrate, temperature, humidity, etc. In general, the type A trichothecenes have been most frequently associated with the following *Fusarium* species: *F. tricinctum*, *F. sporotrichioides*, *F. poe*, *F. equiseti*. On the other hand, type B trichothecenes are usually associated with *F. graminearum* (*Gibberella zeae*), and *F. culmorum*. The type C trichothecenes, containing an additional epoxide function at the 7,8- or 9,10-positions, are produced by only a few species. The Type D trichothecenes, i.e., those containing the macrocyclic ring between the 4,15-positions, are produced by several fungal genera, including *Myrothecium* and *Stachybotrys*. Of these, the most important from an animal and human health standpoint, is *Stachybotrys atra*. The relationships between trichothecenes and fungal species are summarized in Table 13.

The taxonomy of the various fungal species is complex and has led to some confusion, particularly regarding the *Fusarium* species responsible for the production of the various trichothecene metabolites. Several excellent descriptions are now available of the *Fusarium* spp. and the metabolites produced by them (Nelson et al., 1983; Ueno, 1983; Marasas et al., 1984; Ichinoe et al., 1985; Joffe, 1986).

II.2.2 Ecology of trichothecene-producing fungi

Fusarium species are widely distributed throughout the environment. There are over 50 recognized species commonly occurring in the soil (soil fungi), many of which are pathogenic to crop plants. As a result, there are a number of frequently encountered plant diseases (wilts, blights, rots), such as *F. graminearum* blight of wheat and barley (Akakabibyo in Japanese), pink ear rot of corn, pink scab (tombstone kernels) of wheat, *Fusarium* snow

Table 13. The relationship between trichothecenes and fungal species

Trichothecenes	Fungal species	References
Type A		
T-2, HT-2, DAS, NS	*Fusarium tricinctum*	Joffe & Yagen (1977)
	F. sporotrichioides	Scott et al. (1980)
	F. poae	Marasas et al. (1984)
	F. acuminatum	Ichinoe et al. (1985), Rabie et al. (1986)
DAS	*F. equiseti*	Brian et al. (1961)
	F. semitectum	Suzuki et al. (1980) Greenhalgh et al. (1984)
Type B		
DON, 3-AcDON	*F. graminearum*	Yoshizawa & Morooka (1973)
NIV, F-X	*Gibberella zeae* (anamorph)	Marasas et al. (1984) Ichinoe et al. (1985)
DON, 3-AcDON	*F. culmorum*	Marasas et al. (1979b) Chelkowski et al. (1984)
Trichothecin	*Trichothecium roseum*	Freeman & Morrison (1949) Ishii et al. (1986)
Type C		
Baccharin	*Baccharis megapotamica* (higher plant)	Kupchan et al. (1976)
Type D		
Roridin A, D, E	*Mycothecium roridum*	Bohner et al. (1965)
Verrucarin J	*M. verrucaria*	Harrach et al. (1981)
Satratoxin G, H	*Stachybotrys atra*	Jarvis et al. (1986)

blight, etc. *Fusarium graminearum*, which produces DON and NIV, is a most important fungus.

II.2.3 Natural occurrence

It has become apparent in the past few years that whenever a trichothecene-producing *Fusarium* species parasitizes a crop,

food, or animal feed, it is highly probable that the metabolites of the trichothecene will be found as contaminants. The chance of detecting the metabolite is then clearly a function of the efficiency of the sampling procedure and the capabilities of the analytical methods used (most importantly, the detection limit).

Because of its toxicity, analytical procedures for T-2 toxin were developed first, and consequently early surveys for trichothecenes tended to concentrate on T-2 toxin. However, it soon became apparent that other trichothecenes, in particular, DON, NIV, and DAS, were more frequent contaminants of food and animal feed than T-2 toxin. As improved analytical procedures for these mycotoxins became available, surveys were conducted and additional data on the occurrence of these metabolites were published. In reviewing this record, it should be recognized that the methodology used in developing the data was quite variable, so that only broad generalizations with respect to incidence/level may be drawn. In the vast majority of cases, no evidence was included indicating that methods used were rigorously tested, that quality assurance programmes were in place, and that confirmation of identity was adequately obtained. Much of the available survey data on the natural occurrence of trichothecenes in raw agricultural commodities, food, and animal feed is summarized in Tables 14–17. Reports in which only a few samples were analysed have not been included.

II.2.3.1 Agricultural products

(a) T-2 Toxin

One of the first trichothecenes to be implicated in an episode of mouldy corn toxicosis was T-2 toxin; in 1972, it was reported that T-2 toxin at the level of 2 mg/kg was present in mouldy corn involved in lethal toxicosis in dairy cattle (Hsu et al., 1972). This event, along with increasing information regarding the acute toxicity of T-2 toxin, prompted considerable efforts to develop methods of analysis for T-2 toxin and the analysis of a wide range of agricultural commodities (Table 14). Only occasional samples were found to contain T-2 toxin (incidence well below 10% in most cases), most frequently at levels < 0.1 mg/kg. Usually, other trichothecenes were also found (Vesonder, 1983). On the other

Table 14. Natural occurrence of T-2 toxin in raw agricultural commodities

Commodity	Levels (mg/kg)	Incidence (+ve/total)	Country	Reference
Corn	0.5-5.0	(5/150)	Hungary	Szathmary (1983)
	0.080-0.65	(9/118)	Taiwan	Tseng et al. (1983)
	0.01-0.2	(13/20)	New Zealand	Hussein et al. (1989)
Feed	0.05-5.0	(28/464)	Hungary	Szathmary (1983)
Oats	0.01-0.05		Finland	Ylimacki et al. (1979)
Peanuts	0.63-38.89	(6/87)	India	Bhavanishankar & Shantha (1987)
Rice		(3/64)	Egypt	Abdel-Hafez et al. (1987)
Sorghum	1.67-15.0	(4/84)	India	Bhavanishankar & Shantha (1987)
Wheat	2.0-4.0	(3/12)	India	Bhat et al. (1989)

hand, there have been isolated reports of the finding of rather high levels of T-2 toxin, e.g., the finding of 25 mg T-2 toxin/kg in barley (Puls & Greenway, 1976), and 38.9 mg T-2 toxin/kg in peanuts (Bhavanishankar & Shantha, 1987). These findings, as well as reports from India of the presence of T-2 toxin in safflower seed and sweet corn (Ghosal et al., 1977), and sorghum (Ruckmini & Bhat, 1978), and from Italy of its presence in barley, corn feed, oats, rice, and wheat (Cirilli, 1983) need to be further investigated.

DON/NIV: Deoxynivalenol (DON) and nivalenol (NIV) have been found to be the most frequent trichothecene contaminants of agricultural crops throughout the world (Table 15). Extensive survey data indicate the common occurrence of these mycotoxins, particularly in corn and wheat, at levels usually below 1 mg/kg (Vesonder, 1983; Pohland & Wood, 1987; Jelinek et al.,1989). Perhaps the most extensive survey, and the one giving the most accurate picture of the global occurrence of DON and NIV, was reported recently by Tanaka et al. (1988).

Table 15. Natural occurrence of DON and NIV in raw agricultural commodities

Commodity	Toxin	Levels (mg/kg)[a]	Incidence (+ve/total)	Country	Reference
Barley	DON	t-40.4	(19/25)	Japan	Kamimura et al. (1981)
	NIV	t-37.9	(19/23)		
(unpolished)	DON	0.004-0.508	(26/28)	Korea Republic of	Lee et al. (1985)
	NIV	0.017-3.0	(28/28)		
(polished)	DON	0.008-0.043	(5/6)		
	NIV	0.085-0.328	(5/6)		
	DON	0.006-2.14	(34/49)	Norway	Sundheim et al. (1988)
	NIV	0.013-1.56	(49/49)		
	DON	0.02-0.36	(34/87)	United Kingdom	Gilbert et al. (1983b)
Corn	DON	1-20	(19/60)	Austria	Lew et al. (1979)
	DON	0.15-0.82	(9/9)	Canada	Scott et al. (1981)
	DON	0.36-12.7	(100%)	China	Qiujie et al. (1988)
	NIV	0.054-2.67	(100%)		
	DON	0.02-0.3	(11/20)	New Zealand	Hussein et al. (1989)
	DON	t-15.8	(20/72)	Transkei	Thiel et al. (1982)
	NIV	t-1.41	(43/72)		
	DON	0.1-0.3	(37)	United Kingdom	Gilbert et al. (1984)
	DON	0.5-10.7	(24/52)	USA	Vesonder et al. (1978)
	DON	0.1-2.47	(93/198)	USA	Wood & Carter (1989)
Oats	DON	20		Germany, Federal Republic of	Bauer et al. (1980)
	DON	0.02-0.1	(1/6)	United Kingdom	Gilbert et al. (1984)
Rye	DON	0.003	(1/5)	Korea Republic of	Lee et al. (1985)
	NIV	0.046-0.114	(5/5)		
Wheat	DON	0.01-4.3	(51/52)	Canada	Scott et al. (1981)

Table 15 (*continued*)

Commodity	Toxin	Levels (mg/kg)[a]	Incidence (+ve/Total)	Country	Reference
Wheat	DON	0.06-8.53	(24/52)	Canada	Trenholm et al. (1981)
	DON	0.02-1.32	(55/199)	Canada	Osborne & Willis (1984)
	DON	1.0	(1/36)	Denmark	Hald & Krogh (1983)
	DON NIV	t-4.7 t-7.8	(15/18)	Germany, Federal Republic of	Bauer et al. (1980)
	DON	0.008-3.19	(32/53)	Norway	Sundheim et al. (1988)
	NIV	0.015-0.887	(53/53)		
	DON	0.02->0.5	(57/148)	United Kingdom	Gilbert et al. (1984)
	DON	0.12-5.5	(31/33)	USA	Hagler et al. (1984)
	DON	0.2-9	(54/57)	USA	Eppley et al. (1984)
	DON	0.1-2.65	(133/247)	USA	Wood & Carter (1989)
Animal feed	DON	0.1-41.6	(274/342)	USA	Cote et al. (1984)
	DON	<0.4-40		USA	Vesonder (1983)

[a] t = trace.

This survey involved the analysis of 500 samples of cereal grains from 19 countries (Table 16) using a single analytical method.

It was found that 45–50% of random samples contained both DON and NIV, barley being most frequently contaminated. Corn, although less frequently contaminated, contained the highest average amounts of NIV; of all the cereals examined, wheat was the most heavily contaminated with DON. There were clear regional differences, not totally predictable, in the relative quantities of DON and NIV. For example, in Canada and the USA, NIV was only rarely encountered whereas, in Japan, NIV was more frequently encountered than DON. Even within a

Table 16. Natural occurrence of DON and NIV: worldwide survey

Commodity	DON		NIV	
	(Mean, ng/g)	(% +ve)	(Mean, ng/g)	(% +ve)
Barley	149	75	401	76
Corn	402	20	766	16
Oats	115	22	438	26
Rice	0		22	22
Rye	183	33	47	33
Sorghum	0		91	9
Soybean	0		0	
Wheat	488[a]	39	127	50
Others[b]	135	44	3	6
Total:	292	45	267	48

[a] A Beijing wheat sample containing 6 644 ng/g was excluded in calculating this mean.
[b] Wheat flour, 7; rye flour, 1; spice, 3; sesame, 7.

country there were clear differences between regions; for example, in southern Japan, NIV was more frequently encountered than DON, whereas in northern Japan the reverse was true. Similarly, levels of DON were generally lower in western than in eastern Canada (Jelinek et al., 1989).

The data indicate that crops parasitized by *Fusarium* species will probably be contaminated with these mycotoxins. In wheat, for example, there is an excellent correlation between the DON contamination level and the percentage of mouldy kernels, the percentage of total defects, and the degree of scab damage (Eppley et al., 1984; Shotwell et al., 1985). It has been suggested that crop rotation might be a factor, i.e., planting wheat after corn tends to increase the DON levels in the resulting wheat crop (Teich & Hamilton, 1985). Recently, it has been shown that the mean concentration of DON in wheat declines quite significantly in the 2-week period immediately preceding harvest (Scott et al., 1984).

(*b*) *Miscellaneous trichothecenes*

There have been occasional reports of trichothecenes other than those discussed above (T-2 toxin, DON, NIV) in agricultural products, in particular, DAS, 15-acetyldeoxynivalenol (15-ADON), and 3-acetyldeoxynivalenol (3-ADON) (Table 17). Most of these reports involve corn. There is also an unconfirmed report of the finding of T-2 toxin, DAS, and trichothecolone as well as the fatty acid esters of trichothecolone, scirpenetriol, and T-2 tetraol in banana fruit, which needs further investigation (Chakrabarti & Ghosal, 1986). In 1982, over 100 out of a flock of 1200 ewes on a Hungarian farm died after ingestion of bedding straw highly infected with *Stachybotrys atra*. TLC, HPLC, and MS analysis of the methanol extract of the straw revealed

Table 17. Natural occurrence: miscellaneous trichothecenes in agricultural products

Commodity	Toxin	Level (mg/kg)	Incidence (+ve/ total)	Country	Reference
Corn	15-ADON	0.113 ave.		China	Qiujie et al., (1988)
	3-ADON	0.495 ave.		China	Qiujie et al. (1988)
	DAS	31.5		Germany, Federal Republic of	Siegfried (1977)
	DAS	0.01-0.9	(6/20)	N.Zealand	Hussein et al. (1989)
	15-ADON	0-7.9		USA	Abbas et al. (1986)
Peanuts	DAS	0.41-2.03	(7/87)	India	Bhavanishankar & Shantha (1987)
Wheat flour	ADON	0.6-2.4	(4/12)	India	Bhat et al. (1989)
Bedding straw	satra-toxin G		not estimated	Hungary	Harrach et al. (1983)
	satra-toxin H		not estimated	Hungary	Harrach et al. (1983)

the presence of the macrocyclic trichothecenes, satratoxins G and H (Harrach et al., 1983).

The macrocyclic trichothecenes, such as satratoxin H, verrucarins B and J, and trichoverrins A and B, were also detected in air dust collected from a house in Chicago, where the occupants were subjected to a variety of recurring maladies including colds, dermatitis, and others (Croft et al., 1986). These data indicated a possible airborne outbreak of macrocyclic trichothecene-induced toxicosis.

Undoubtedly, as good analytical methods become available, these trichothecenes will be found more frequently.

II.2.3.2 Trichothecenes in human foodstuffs

Given the widespread occurrence of trichothecenes in agricultural products, in particular DON and NIV, it is not surprising to find the compounds in human foodstuffs (Table 18). The vast majority of the confirmed cases of contamination of foodstuffs by trichothecenes involve DON in wheat or wheat products. Overall, the finding of DON in human foodstuffs at concentrations >1 mg/kg has been rare. NIV has been detected in human foodstuffs, particularly in Japan, where an effort was made to develop and apply analytical methods capable of determining both DON and NIV.

The realization that trichothecene contamination of cereal grains was not uncommon prompted a series of studies on the fate of these compounds during the normal processing of such grains into consumer food products. These studies can be summarized as follows:

In the case of corn, Collins & Rosen (1981) demonstrated that, during wet milling, roughly 66% of the T-2 toxin originally present was removed in the steeping water, about 4% remained in the starch, and the remainder was distributed evenly in the germ, gluten, and fibre fractions (a result clearly predictable in view of the considerable water solubility of T-2 toxin). The same general observation was made about the fate of DON during the wet milling of corn (Scott, 1984b). During the dry milling of corn,

Table 18. Natural occurrence of DON and NIV in commercial foods

Commodity	Toxin	Level (mg/kg)[a]	Incidence (+ve/total)	Country	Reference
Barley flour (parched)	DON	0.027-0.085	(6/6)	Japan	Yoshizawa & Hosokawa (1983)
	NIV	0.037-0.19	(6/6)		
Barley flour	DON	0.008-0.039	(5/6)		Tanaka et al.. (1985b)
	NIV	0.013-0.041	(6/6)		
Barley (pressed)	DON	0.003-0.05	(10/14)		Tanaka et al.. (1985a)
	NIV	0.008-0.033	(13/14)		
Barley products	DON	t-0.26	(27/147)		Kamimura et al. (1981)
	NIV	0.006-0.28			
Corn meal	DON	0-0.25	(45/50)	USA	Trucksess et al. (1986a)
Corn flour	DON	(0.18 ave.)	(27)	Canada	Scott et al.. (1984)
meal	DON	(0.1 ave.)	(35)		
products	DON	0.011-1.25	(7/7)		
Popcorn	DON	0.012-0.25	(7/7)	Japan	Tanaka et al.. (1985b)
Job's tear	DON	0.048-0.5	(2/12)		
	NIV	0.003-0.92	(11/12)		
Potatoes	DON		(4/17)	Canada	El Banna et al. (1984)
Rye flour	DON	(0.12 ave.)	(3)	Canada	Scott et al.. (1984)
bread	NIV	(0.058 ave.)	(4)		
Wheat flour	DON	(0.4 ave.)	(43)		
	DON	0.11-0.69	(5/5)	China	Ueno et al. (1986)
	DON	0.43-4.89	(9/12)	India	Bhat et al. (1989)
	NIV	0.03-0.1	(2/12)		
	DON	0.002-0.239	(26/36)	Japan	Tanaka et al.. (1985b)
	NIV	0.004-0.084	(12/36)		
	DON	0-0.46	(44/50)	USA	Trucksess et al. (1986a)
Breakfast cereals	DON	(0.086 ave.)	(36)	Canada	Scott et al.. (1984)
Bread	DON	(0.08 ave.)	(21)		
Baby cereal	DON	(0.043 ave.)	(30)		
Crackers	DON	(0.27 ave.)	(20)		
Cookies	DON	(0.12 ave.)	(25)		
Breakfast cereals	DON	0-0.53	(35/60)	USA	Trucksess et al. (1986a)
Bread	DON	0-0.24	(20/25)		
Baby foods	DON	0-0.09	(14/39)		
Snack foods	DON	0-0.45	(25/44)		
Bread	DON	0.013-0.24	(39/45)	USA	Wood & Carter (1989)

[a] t = trace.

the major portion of contaminating DON was found in the germ meal, which is used for animal feed. For any significant contamination of grits, the corn used in the manufacture would have to be highly contaminated (Gilbert et al., 1983a).

In the case of wheat, several studies have clearly demonstrated that cleaning and milling are not effective in completely removing DON (Hart & Baselton, 1983; Scott et al., 1983, 1984a; Young et al., 1984; Seitz et al., 1985). The same was generally true for NIV (Lee et al., 1987). Normal cleaning of wheat reduced average DON contamination levels by 6–19% (Abbas et al., 1985). The remaining DON, after milling, is distributed in all fractions (bran, shorts, reduction flour, break flour), the greatest amounts being found in the bran and the smallest amounts in the flour, depending on the level of contamination of the wheat itself. In a typical study, milling of whole wheat contaminated at the 2 μg/g level, resulted in approximately 65% of the DON in the flour and 35% in the bran, red dogs, and shorts; i.e., there was little reduction in the DON concentration in the flour after milling (Hart & Baselton, 1983).

It has been found that the further processing of flour into baked or cooked products results in variable DON losses. The overall range of reduction in DON levels from uncleaned wheat to bread was 24–71% (Abbas et al., 1985). No reduction in DON levels was found in the preparation of Egyptian bread (El-Banna et al., 1983) or cookies from hard wheat flour (Scott et al, 1983), illustrating the importance played by the conditions of processing. Kamimura et al. (1979) showed that bread and noodles prepared under conditions simulating commercial processes retained about 50% of the trichothecenes that had been previously added to the wheat flour. The trichothecene content of Japanese noodles was reduced by 30% in the boiling process.

Similarly, these authors found that 30% of NIV and DON was extracted from naturally contaminated wheat by water, while there was a 50% or more reduction in trichothecene levels in bread made from the same flour. The preparation of Chinese noodles, where sodium carbonate is used as an ingredient, results in an even greater loss of DON than is observed in the preparation of Japanese noodles (Scott et al., 1984b; Nowicki et al.,

1988). Similarly, Young et al. (1984) observed a decrease of up to 35% in the production of cookies from biscuit and cake flours; in the process studied, the batter contained ammonium carbonate. Finally, it has been found that, during the baking of bread, 3–13% of DON is converted into an isomer, iso-DON (Greenhalgh et al., 1984).

There have been some studies on possible detoxification procedures for corn and wheat contaminated with trichothecenes; agents studied include chlorine, ammonia (Young et al., 1984) and aqueous bisulfite (Young et al., 1986). None of these processes are currently commercially feasible.

There has been some concern about the transmission of the trichothecenes, particularly DON, to milk. Studies have shown that milk is only a minor excretion route in the lactating dairy cow; thus, the possibility that DON might contaminate milk and milk products is remote (Prelusky et al., 1984).

II.3 METABOLISM

II.3.1 Absorption and tissue distribution

II.3.1.1 Animal studies

In general, trichothecenes absorbed from the alimentary tract are evenly distributed in many tissues and organs, without significant accumulation in specific organs. However, at present, distribution studies have been limited mainly to T-2 toxin, and the metabolism in animals of other naturally contaminating trichothecenes, such as DON, NIV, and DAS, remains to be elucidated.

In 6-week-old broiler chicks fed with a ration containing T-2 toxin at 2 mg/kg for 5 weeks and then intubated with a single dose of ^{3}H-T-2 toxin at 0.5 mg/kg body weight, the radioactivity reached a maximum concentration in most tissues, 4 h after dosing; exceptions were the muscle, skin, and bile, in which the maximum level was reached after 12 h (Chi et al., 1978b). After 48 h, chicks contained the equivalent of 39 μg T-2 toxin and/or its metabolites/kg in the muscle, and 40 μg/kg in the liver, as calculated on the basis of the specific activity of the radiolabelled T-2 toxin administered.

In chicken organs, 18 h after intraperitoneal injection of T-2 toxin (3.5 mg/kg), considerable amounts of T-2 metabolites were found in the liver (1370 μg 3′-hydroxy-H-T-2 toxin/kg). Smaller amounts of H-T-2 toxin, T-2 triol, and other metabolites were detected in the lungs (Visconti & Mirocha, 1985).

In weanling crossbred pigs (7.5–9.5 kg body weight) intubated with 0.1 or 0.4 mg ^{3}H-T-2 toxin/kg body weight, the percentage of administered radioactivity (18 h after dosing) was 0.7% in the muscle, 0.29–0.43% in the liver, 0.08% in the kidney, and 0.06–0.14% in the bile (Robison et al., 1979b). In pigs intubated with 0.1 mg of the toxin/kg body weight, the calculated residue levels for T-2 toxin and/or its metabolites, based on the specific radioactivity of the tissues, were as follows: muscle, 3.1 μg/kg;

fat, 0.49 μg/kg; liver, 13.8 μg/kg; and kidney, 15.9 μg/kg. The corresponding residue levels for T-2 toxin in the tissue of another pig intubated with 0.4 mg of the toxin/kg body weight were 11.5 μg/kg in the muscle, 37.7 μg/kg in the liver, and 61.4 μg/kg in the kidney.

Four hours after intravenous administration of T-2 toxin in swine, the greatest amount of radioactivity was located in the gastrointestinal tract (15–24% of the dose), and 4.7–5.2% of the dose was found in the remaining tissues, among which the muscle and the liver accounted for 2.9–3.2% and 0.7–1.7% of the dose, respectively (Corley et al., 1986).

The fate and distribution of ^{3}H-T-2 toxin was studied in guinea-pigs (Pace et al., 1985). Except for the large intestine and bile, the radioactivity had peaked by 30 min and rapidly declined, with no measurable long-term accumulation. In general, the distribution pattern in the guinea-pig during the first 12–24 h paralleled the distribution found in the chicken and swine.

The metabolic fate of T-2 toxin was investigated in a lactating Jersey cow weighing 375 kg, after daily oral administration, by capsule, of unlabelled toxin at 180 mg/day for 3 days, followed by administration of ^{3}H-T-2 toxin at 156.9 mg (Yoshizawa et al., 1981). Although almost all of the administered dose was eliminated in 72 h, appreciable levels of tritium still remained in the bile, liver, and kidney (equivalent, repectively, to 27.2, 18.5, and 13.9 μg/kg ^{3}H-T-2 toxin) 3 days after dosing. These levels are higher than in whole blood (13.3 μg/kg) and plasma (10.2 μg/kg) and in other tissues, including the spleen (9.4 μg/kg) heart (10.1 μg/kg), mammary gland (11.3 μg/kg), ovaries (10.7 μg/kg), muscle (8.8 μg/kg), and fat (4.7 μg/kg).

Experimentally derived relationships between the residue of radioactivity in animal tissues or plasma and the toxin levels in the feed, calculated on the basis of the amount of ^{3}H-T-2 toxin administered are summarized in Table 19 (Yoshizawa et al., 1981).

The tissue/feed ratio of tritium in the edible tissues of the cow, 72 h after dosing, ranges from 0.0003 to 0.0006. These figures are 5–10% of those for swine, 18 h after dosing. The tissue/feed

Table 19. The relationship between the level of ^{3}H-T-2 toxin in the feed or the tritium residues in plasma and tritium levels in the edible tissues of the cow, chick, and pig[a]

Tissue	Animal	Time after dosing (h)[b]	Feed level (mg/kg)[c]	Tissue level (μg/kg)[d]	Tissue/feed ratio	Tissue/plasma ratio[e]
Muscle	Cow	72	31.38	8.8	0.0003	0.863
	Chick	24	1.26	17.3	0.0137	1.000
			5.0	59.2	0.0118	0.938
			18.95	228.6	0.0121	0.875
	Pig	18	1.25	3.1	0.002	0.775
Heart	Cow	72	31.38	10.1	0.0003	0.991
	Chick	24	1.26	13.7	0.011	0.792
			5.0	49.4	0.010	0.783
			18.95	207.7	0.011	0.795
	Pig	18	1.25	3.9	0.003	0.975
Liver	Cow	72	31.38	18.5	0.0006	1.863
	Chick	24	1.26	34.0	0.0270	1.965
			5.0	107.3	0.0215	1.700
			18.95	431.0	0.0227	1.649
	Pig	18	1.25	13.8	0.011	3.450
Milk	Cow	72	31.38	11.4	0.0004	1.118

Table 19 (*continued*)

[a] From: Yoshizawa et al. (1981).
[b] Animals were intubated with a single dose of ^{3}H-T-2 toxin.
[c] Estimates of feed levels were based on the assumption that each animal would consume the following amount of feed daily: cow, 5 kg; chick, 100 g; pig, 600 g.
[d] Values were calculated from residual tritium levels in the edible tissues of animals given ^{3}H-T-2 toxin and were expressed as equivalents of 3H-T-2 toxin (μg/kg).
[e] The plasma levels were 10.2 μg/kg equivalents of T-2 toxin in the cow, and 17.3, 63.1, and 261.3 μg/kg equivalents of T-2 toxin in the chick at feed levels of 1.26, 5, and 18.95 mg/kg, respectively. The whole-blood level of residual tritium in the pig was 4 μg/kg equivalents of T-2 toxin.

ratio for chickens, is higher than those in the cow and pig, ranging from 0.010 to 0.014 for the muscle and heart, and from 0.021 to 0.027 for the liver, regardless of T-2 toxin dosage. The tissue/plasma ratios of T-2 toxin metabolites, ranging from 0.8 to 1 in the muscle and heart, and from 1.6 to 3.5 in the liver, are independent of animal species, of T-2 toxin dosage, and of time after dosing. The milk/plasma ratio of radioactivity in a cow treated with ^{3}H-T-2 toxin increased linearly for 24 h and thereafter ranged from 1 to 1.3.

II.3.2 Metabolic transformation

The *in vivo* metabolic transformation of trichothecenes in animals is reported in Table 20. The trichothecene metabolites produced are less toxic than the corresponding parent toxins. Both de-epoxidation and glucuronidation, in particular, are associated with remarkable detoxification of trichothecenes.

In *in vitro* studies, HT-2 toxin was found to be the sole metabolite of T-2 toxin in the microsomes of the liver, kidney, and spleen of various animals (Ohta et al., 1977, Johnsen et al., 1986). The reaction of hepatic microsomes of different species in nmol/mg protein per 10 min were: rabbit, 3044; human, 331; mouse, 75; chicken, 55; rat, 36; and guinea-pig, 14. Yoshizawa et al. (1984, 1986) have proposed an *in vitro* metabolic pathway for T-2 toxin that includes the hydrolysates at the C-4, C-8, and C-15 positions, and the hydroxylation at the C-3′ position by liver homogenates from the rat, mouse, or monkey. This hydrolytic transformation was also observed in the hepatic homogenates of the rabbit, pig, and cow. However, HT-2 toxin was the sole metabolite in the homogenate of the chicken, suggesting species differences in the metabolic pathway of T-2 toxin (Yoshizawa & Sakamoto, 1982). In addition to the metabolites above, glucuronide conjugates were formed in a study using isolated perfused rat livers and T-2 toxin and DAS (Gareis et al., 1986). Various de-epoxidation metabolites were found by incubating intestinal and rumen microbes with T-2 toxin and its metabolites (Yoshizawa et al., 1985a; Swanson et al., 1987), DON (King et al., 1984; Côté et al., 1986; Swanson et al., 1987), and DAS (Swanson et al., 1987).

Table 20. *In vivo* (metabolic) transformation of trichothecenes in animals

Animal	Trichothecene	Transformation reaction	Reference
Chicken	T-2 toxin	hydrolysis, 3′-hydroxylation, acetylation	Yoshizawa et al. (1980b); Visconti & Mirocha (1985)
Cow	T-2 toxin	hydrolysis, 3′-hydroxylation, 7′-hydroxylation, de-epoxidation	Yoshizawa et al. (1981, 1982a,b) Pawlosky & Mirocha (1984) Chatterjee et al. (1986)
	DON	de-epoxidation, glucuronide conjugation	Yoshizawa et al. (1986) Côté et al. (1986)
Pig	T-2 toxin	hydrolysis, 3′-hydroxylation, de-epoxidation, glucuronide conjugation	Corley et al. (1985, 1986)
	DAS	hydrolysis	Bauer et al. (1985)
Sheep	DON	glucuronide conjugation	Prelusky et al. (1985)
Rat	DON T-2 metabolites DAS	de-epoxidation de-epoxidation hydrolysis, de-epoxidation	Yoshizawa et al. (1983) Yoshizawa et al. (1985a,b) Sakamoto et al. (1986)
Guinea-pig	T-2 toxin	hydrolysis, 3′-hydroxylation	Pace et al. (1985)
Dog	T-2 toxin	hydrolysis, glucuronide conjugation	Sintov et al. (1987)

II.3.3 Excretion

II.3.3.1 Animal studies

The kinetics of T-2 toxin were determined in pigs and cattle (Beasley et al., 1986), and dogs (Sintov et al., 1986). Mean elimination phase half-lives were 13.8 and 17.4 min, and mean apparent specific volumes of distribution were 0.366 and 0.375 litre/kg in intra-aortally dosed pigs and intravenously dosed calves, respectively. In dogs, the following mean parameters were determined after intravenous administration of T-2 toxin and HT-2 toxin, respectively: half-life 5.3 and 19.6 min, clearance 0.107 and 0.167litre/min per kg, and volumes of distribution 0.86 and 4.47 litre/kg.

The half-life of elimination of DON in sheep, ranged from 100 to 125 min following oral administration, and it took 20–30 h to clear from the system. Glucuronidation after intravenous or oral administration of DON appeared to occur quite efficiently (iv, 21%; oral, 75%), with elimination half-lives of (150–200 min and 6.1–7.1 h, respectively). These were considerably longer than those of the parent toxin (Prelusky et al., 1985).

II.3.3.2 Excretion in eggs and milk

Radioactivity was transmitted into the eggs from laying hens that had been intubated gastrically with a single or several doses of ^{3}H-T-2 toxin (Chi et al., 1978a). In birds dosed singly with 0.25 mg T-2 toxin/kg body weight, maximum residues in the eggs occurred 24 h after dosing; the yolk contained 0.04% of the total dose and the white contained 0.13%. In birds dosed with 0.1 mg T-2 toxin/kg body weight per day for 8 consecutive days, the radioactivity in the egg accumulated until the 5th day of dosing, remained unchanged until the last day of dosing, and rapidly decreased thereafter. Assuming that the birds weighing 1.6 kg consumed 100 g of the diet containing 1.6 mg toxin/kg daily, the residues (T-2 toxin and/or its metabolites) in such contaminated eggs would be about 0.9 μg/egg.

Transmission of DON was studied in the eggs and meat of chickens (El Banna et al., 1983; Prelusky et al., 1987). Following

a single oral dose of ^{14}C DON, maximum radioactivity, which occurred in the first eggs laid after dosing (within 24 h), amounted to 0.087% of the dose: levels dropped rapidly in later eggs.

In a pregnant Holstein cow (third trimester) intubated daily with 180 mg T-2 toxin for consecutive days (the toxin level corresponded to a concentration of 50 mg/kg in the feed), milk samples taken on the 2nd, 5th, 10th, and 12th days of intubation contained T-2 toxin concentrations ranging from 10 to 160 μg/kg (Robison et al., 1979a). Transmission of DON-1 (de-epoxydeoxynivalenol) to milk was confirmed in lactating dairy cows (Côté et al., 1986; Yoshizawa et al., 1986). Fresh and conjugated DON were also present in cow's milk following administration of a single oral dose of 920 mg DON, but only extremely low amounts (< 4 μg/litre) were detected (Prelusky et al., 1984).

II.4 EFFECTS ON ANIMALS

II.4.1 Field observations

In Hungary and other central European countries, pyoseptic-aemia has been reported sporadically, in the past, in horses after ingestion of mouldy hay and straw. This disease was characterized by haemorrhages of the intestine and muscles, severe diarrhoea, and death. *Bacterium pyosepticum viscosum* was detected in 1929 in the excreta, and the equine disease was diagnosed as a pyosepticaemia (Forgacs, 1965; Danko & Szerafin, 1976). After the discovery of toxigenic *Stachybotrys atra* and its metabolites, such as satratoxins G and H (Eppley & Bailey, 1973), the disease was presumed to be the same as stachybotryotoxicosis (Danko & Szerafin, 1976).

A field outbreak involving the death of 20% of a dairy herd was associated with prolonged ingestion of a diet containing 60% mouldy corn infested with *F. tricinctum*. The concentration of T-2 toxin in the feed was approximately 2 mg/kg dry weight (Hsu et al, 1972). The lesions in the cattle included extensive haemorrhages on the serosal surface of the internal viscera. An outbreak of haemorrhagic syndrome in cows was associated with commercial feed containing T-2 toxin or T-2-like toxin (concentration not determined). The affected animals showed an extremely prolonged prothrombin time. Necropsy findings in 2 adult cows were marked serosal, mucosal, and subcutaneous haemorrhages (Hibbs et al., 1975).

An outbreak of a disease, observed in poultry (ducks, geese), horses, and pigs, was associated with mouldy barley containing T-2 toxin at approximately 25 mg/kg (Greenway & Puls, 1976). Pigs fed the suspect barley exhibited signs of feed refusal, vomiting, and diarrhoea. The horses became depressed and salivated excessively. The lesions in the geese included necrosis of the mucosa of the oesophagus, proventriculus, and gizzard. No pathological lesions were described in other animals.

DON was isolated from a batch of maize that had caused vomiting in pigs (Vesonder et al., 1973).

Equine leukoencephalomalacia reported from South Africa (Kellerman et al., 1972; Marasas et al., 1979a; Pienaar et al., 1981) and bean-hull toxicosis reported in horses in Hokkaido, Japan (Konishi & Ichijo, 1970) appear to be very similar diseases with nervous signs and hepatopathy as the major components. The signs in these diseases were quite different from those caused by trichothecenes, though some fungal isolates from samples of bean-hulls produced the trichothecenes, T-2 toxin, and neosolaniol (Ueno et al., 1972). *F. moniliforme* was considered to be the causative fungus (Haliburton et al., 1979), but none of its metabolites, including moniliformin, have been established as the cause of the disease (Kriek et al., 1977).

Reports of field outbreaks of animal toxicoses associated with trichothecene-contaminated feed are summarized in Table 21.

II.4.2 Effects on experimental animals

II.4.2.1 General toxic effects

LD_{50} values for certain trichothecenes in several experimental animal species are summarized in Table 22 (Ueno et al., 1983). The oral LD_{50} for T-2 toxin was 10.5 mg/kg body weight in mice, 3.06 mg/kg in guinea-pigs, 5.2 mg/kg in rats, and 6.1 mg/kg in trout. The LD_{50} values for T-2 toxin in different species vary, but not greatly.

The LD_{50} values of fusarenon-X were compared using different routes of administration. The LD_{50}s (mg/kg) in mice were 3.4 (iv), 3.4 (ip), 4.2 (sc), and 4.5 (oral). These data indicate that the acute toxicity estimated for a single administration did not differ markedly when the toxin was administered by different routes (Table 20) (Ueno et al., 1971).

Similar data were obtained with DON and acetyl-DON. An interesting finding was that the ratio of the maximum lethal dose to the minimum lethal dose was approximately two, indicating a sharp dose-response curve for lethality. No marked differences in acute toxicity were observed between treated male and female animals.

Table 21. Field observations on animal toxicosis caused by trichothecenes

Mycotoxin (concentration in feed)	Sample	Animal	Signs & lesions	Reference
T-2 toxin (2 mg/kg)	mouldy corn	cattle	extensive haemorrhages (20% died)	Hsu et al. (1972)
T-2 toxin (25 mg/kg)	mouldy barley	poultry, horse, pig	necrosis of mucosa in the proventriculus and oesophagous of geese	Greenway & Puls (1976)
T-2 toxin	commercial feed	cow	haemorrhages	Hibbs et al. (1975)
DAS	maize	pig	vomiting	Vesonder et al. (1973)
T-2 toxin	corn meal	horse	oral lesions, haemorrhages, (6 out of 58 died)	Szathmary (1983)
T-2 toxin	alfalfa	horse	inappetence, listlessness, (13 out of 31 died)	Szathmary (1983)
T-2 toxin		poultry	oral lesions, inappetence, death	Szathmary (1983)
DAS and T-2 toxin	corn	cattle	death	Szathmary (1983)
DAS	oat, sifting	pigeon	emesis, bloody stools	Szathmary (1983)

Table 21 (*continued*)

T-2 toxin (2.5 mg/kg)	chicken feed	broiler chicken	inflammations, "atrophies"	Szathmary (1983)
DAS (150-300 mg/kg)		cattle, pig	haemorrhagic syndrome	Cirilli (1983)
T-2 toxin (50-150 mg/kg)		pig, cattle	bloody stools (swine, cattle) ear necrosis (swine) intestinal lesions (poultry) hepatic lesions (swine)	Cirilli (1983)

Table 22. LD_{50} values (mg/kg) of trichothecenes[a]

Type	Trichothecenes	Mouse				Rat				Guinea-pig		
		iv	ip	sc	oral	iv	ip	sc	oral	ip	sc	oral
A	T-2 toxin		5.2		10.5				5.2			3.06
	HT-2 toxin		9.0									
	Diacetoxyscirpenol (DAS)	12	23.0			1.3	0.75		7.3			
	Neosolaniol		14.5									
	Monoacetoxyscirpenol							0.725				
B	Nivalenol (NIV)	7.3	7.4	7.2	38.9							
	Fusarenon-X	3.4	3.4	4.2	4.5				4.4	0.5	0.1	
	Diacetylnivalenol		9.6									
	Deoxynivalenol (DON)		70.0		46.0							
	3-Acetyldeoxynivalenol		49.0		34.0							
	Trichothecin	300						250				
D	Roridin A	1.0										
	Verrucarin A	1.5	0.5			0.87						
	Verrucarin B	7.0										
	Verrucarin J		0.5									

Table 22 (*continued*)

Type	Trichothecenes	Rabbit (iv)	Cat (sc)	Dog (iv)	Pig (iv)	Duckling (sc)	Day-old chick (oral)	Chick (oral)	Trout (oral)
A	T-2 toxin		0.5		1.21		1.75	4.0	6.1
	HT-2 toxin						6.25		
	DAS	1.0		ca 1.1	0.37			5.0	
	3'-OH HT-2 toxin						8.5		
	15-Acetyl-T-2 tetraol						10.0		
	T-2 tetraol						10.0		
B	Fusarenon-X		5.0			ca 2.0			
	DON					27.0			
	Acetyldeoxynivalenol					37.0			
D	Verrucarin A	0.54							

[a] Adapted from: Ueno et al. (1983) and Ryu et al. (1988).

Newborn animals are more sensitive than adults to the toxic effects of the trichothecenes. For example, the LD_{50} values for toxin given sc (mg/kg body weight) in newborn mice were: T-2 toxin, 0.15; DAS, 0.17; and fusarenon-X 0.23 (Ueno et al., 1973a).

The toxic potency of the trichothecenes varies depending on the modification of side chains in the molecule. The acute lethal toxicity of certain trichothecenes was investigated using a single ip injection in mice and the LD_{50} values (mg/kg body weight) were: verrucarin A and B, 0.5; fusarenon-X, 3.4; NIV, 4.1; T-2 toxin, 5.2; HT-2 toxin, 9.0; diacetylnivalenol, 9.6; neosolaniol (8-hydroxydiacetoxyscirpenol), 14.5; DAS, 23.0; acetyldeoxynivalenol, 49.0; DON, 70.0; and crotocin, 810.0.

Matsuoka et al. (1979) investigated the general effects of fusarenon-X on mice and rats. Fusarenon-X induced hypothermia, but did not induce appreciable behavioural changes in mice. In ether-anaesthetized rats, it caused a rise in blood pressure and a decrease in respiratory rate, but did not induce any significant effects on cardiac rate, the muscle cell membrane, or nerve elements.

The administration of trichothecenes to some animals (rats, mice, and guinea-pigs) induces diarrhoea. The mechanism of this sign was investigated using fusarenon-X and rats (Matsuoka & Kubota, 1981). The ip injection of fusarenon-X in rats caused watery diarrhoea within 36–60 h. At necropsy, 24 h after injection of fusarenon-X, the small intestine was distended, but no blood was found in the lumen of the intestine. The mycotoxin increased the absorption rate of D-xylose from the intestine *in vitro* (Matsuoka & Kubota, 1981). The leakage of intravenously injected Evan's blue dye into the intestine also increased, but the sodium level in the serum decreased. The intestinal villi were shortened and there was extravasation of erythrocytes in the intestinal lamina propria. The diarrhoea induced by ip administration of 1.0 mg fusarenon/kg in male Wistar rats was not mediated by the cyclic nucleotide system as the mycotoxin did not increase the cyclic GMP and AMP contents in the intestinal mucosa (Matsuoka & Kubota, 1987). The permeability of abdominal blood vessels was increased in a dose-dependent

manner in mice given an ip injection of fusarenon-X, and the peak was reached about 8 h after injection. The increased permeability was not mediated by serotonin, histamine, norepinephrine, prostaglandins, leukotrienes, or thromboxanes (Matsuoka & Kubota, 1987).

The effects of fusarenon-X and T-2 toxin on intestinal absorption of monosaccharides were studied in rats. The absorption of 3-*O*-methyl-glucose was reduced 1–3 times after either toxin was injected into the jejunal lumen. Absorption of 3-*O*-methyl-glucose was also reduced after the toxins were given by intravenous injection. Both toxins impaired jejunal function by causing specific damage in the active transport and diffusional movement of monosaccharides (Kumagai & Shimizu, 1988).

Vomiting was one of the most significant signs of trichothecene-induced toxicosis in the cat, dog, pig, and duckling (Ueno, 1980a). T-2 toxin and related trichothecene mycotoxins at doses of 0.1–10 mg/kg induced vomiting (Vesonder et al., 1973; Sato et al., 1975; Yoshizawa & Morooka, 1977; Ueno, 1980b; Matsuoka & Kubota, 1981). The presence of the causal factor, DON, in mouldy corn was established using ducklings as the assay animal (Ueno et al., 1974). In pigs given 0.5 mg/kg body weight by infusion, vomiting commenced 6–7 min after dosing. Emesis or retching occurred at intervals of 2–15 min and lasted from 0.5 to 2 min (Coppock et al., 1985). It is strongly suggested that the mechanism of the vomiting of trichothecenes is their possible action on the chemoreceptor trigger zone (CTZ) in the medulla oblongata (Matsuoka et al., 1979). The iv administration of 0.3 mg fusarenon-X/kg body weight to dogs induced emesis and vomiting, 5–15 min after injection. Vomiting after injection of fusarenon-X was prevented by prior administration of 0.5 mg metoclopramide hydrochloride/kg body weight or 1 mg chlorpromazine hydrochloride/kg.

The cardiovascular effects of the trichothecenes have varied according to such factors as species, dose, and duration of exposure. In acute studies, T-2 toxin given intravenously at 1 mg/kg body weight produced a decline in blood pressure several hours after administration, the reduced blood pressure being accompanied by a decrease in heart rate (Smalley et al., 1970). A single

dose of T-2 toxin administered to guinea-pigs and rabbits resulted in a decrease in systemic blood pressure and a decrease in heart rate (Parker et al., 1984; Wilson, 1984).

Using the *in vitro* bovine ear perfusion system, it was determined that T-2 toxin can cause a dose-dependent vasoconstrictor response in peripheral vasculature, but that the toxin is a less potent vasoactive agent than either histamine or norepinephrine. The presence of known histamine or noradrenergic anatogonist did not affect the response to the toxin (Wilson & Gentry, 1985). T-2 toxin administered systemically produced a marked increase in peripheral vascular resistance in the conscious rat. The cardiac output gradually decreased eventually resulting in cardiovascular collapse and death (Feuerstein et al., 1985).

(a) Swine

The acute and short-term toxicities of T-2 toxin, DAS, and DON were investigated in pigs (Weaver et al., 1978a,b; Coppock et al., 1985). A single dose LD_{50} of T-2 toxin dissolved in ethanol and administered iv was 1.21 ± 0.15 mg/kg body weight in normal, healthy, crossbred pigs weighing from 3 to 50 kg. Soon after administration, emesis was followed by eager consumption of feed, moderate posterior paresis, staggering gait, extreme listlessness, and frequent defecation of normal stools. Between 1 and 6 h, severe posterior paresis, knuckling-over of the rear feet, and extreme lethargy were observed. These signs were followed by severe posterior paresis, frequent falling because of hind-quarter weakness, and the dragging of both rear legs while moving about. Twenty-four hours after administration, the surviving pigs appeared normal. Similar clinical signs were observed in pigs exposed to T-2 toxin through inhalation (Pang et al., 1988). Pathologically, necrosis was present in the epithelial cells of the mucosa and in the crypt cells of the jejunum and ileum, the Peyer's patches of the ileum, the lymphoid elements of the caecum, the lymphoid follicles in the spleen, and the germinal centre of the mesenteric lymph node (Weaver et al., 1978a).

Young pigs were fed with T-2 toxin at 1, 2, 4, or 8 mg/kg standard pig ration for 8 weeks. No statistically significant differences in body weight gain and feed consumption were observed between the treated animals and the controls. Young pigs

refused a ration containing 16 mg T-2 toxin/kg, but not a diet containing 10–12 mg/kg. The no-observed-effect level was estimated to be less than 1 mg/kg, based on differences in body weight gain (Weaver et al., 1978b). In terms of clinical haematological changes, such as haemorrhaging, blood cell counts, serum-enzyme activities, and serum-protein levels, the no-observed-effect level could not be accurately determined, but was higher than 12 mg/kg, based on the weight gain.

The intravenous administration of T-2 toxin to pigs at doses of 4 or 8 mg/kg resulted in a shock syndrome characterized by reductions in cardiac output and blood pressure and increased plasma concentrations of epinephrine, norepinephrine, thromboxane B_2, 6-keto-PGF_{1a} and lactate (Lorenzana et al., 1985). The pigs in the high-dose group produced such signs as persistent vomiting, watery diarrhoea, abdominal straining, cold extremities, coma, and death.

Eighteen white cross-bred female pigs weighing 40–60 kg, immunized against erysepelas, were administered purified T-2 toxin dissolved in 70% ethanol, intravenously, in doses of 0 (5 pigs), 0.6 (5 pigs), 1.2 (1 pig), 4.8 (5 pigs) and 5.4 (2 pigs) mg/kg. The animals administered doses of 4.8 and 5.4 mg/kg died between 5 and 10.5 h later and other groups were killed 12–24 h after treatment. Gross lesions were observed in pigs given 1.2 mg/kg or more and these consisted of oedema, congestion, and haemorrhages of the lymph nodes and pancreas and congestion and haemorrhages of the gastrointestinal mucosa, subendocardium, adrenal glands, and meninges. Histological alterations confirmed the gross lesions. Other lesions were widespread degeneration and necrosis of lymphoid tissue and the surface and crypt epithelium of the intestines. Scattered foci of necrosis were present in the pancreas, myocardium, bone marrow, adrenal cortex, and the tubular epithelium of the renal medulla. Most lesions were dose dependent. The T-2 toxin-induced lesions in the lymphoid and gastrointestinal tract of pigs were similar to those described in other species. The heart and pancreas were additional target organs in pigs (Pang et al., 1987b).

Male, castrated, crossbred, specific pathogen-free pigs (17 controls and 17 treated), 9–11 weeks of age, were used in a study

to characterize the pulmonary and systemic responses to inhaled T-2 toxin (nebulized dose of 9 mg/kg given by endotracheal tube) (Pang et al., 1987a). The animals were exposed to the aerosol in pairs, one animal receiving the toxin, the other acting as a control. From 20 to 30% of the toxin was retained by the pigs (T-2 toxin was mixed with 100–200 μCi technetium for measurement). Five pairs of animals each were killed 1, 3, and 7 days after dosing. Two pairs were designated a 0.33 day group, when one treated pig died and the other was killed in a moribund state, 0–10 h after dosing. Clinically, the T-2 toxin-treated pigs vomited after exposure producing such signs as cyanosis, anorexia, and lethargy. The pigs became laterally recumbent. Alveolar macrophages showed reduced phagocytosis and the blastogenic responses to mitogen were reduced for pulmonary lymphocytes, but not for lymphocytes of the peripheral blood. The lesions in pigs that died included multifocal interstitial pneumonia, necrosis of lymphoid tissue, necrohaemorrhagic gastroenteritis, oedema of gall bladder mucosa, and multifocal areas of necrosis in the heart and pancreas. Inhalation exposure to T-2 toxin produced a clinical and morphological syndrome resembling that produced by intravenously administered T-2 toxin, at doses of 1.2 mg/kg (approximate LD_{50}) or more, as well as death. Furthermore, the lesions produced by the inhaled toxin were more severe.

The acute effects of DAS were studied using single, intravenous doses (range: 0.30–0.48 mg/kg body weight) in 13 crossbred pigs; there were 2 control pigs (Weaver et al., 1978b). Seven of the toxin-exposed pigs developed emesis, frequent defecation, lethargy, staggering gait, and prostration by 10 h leading to death. Severe haemorrhagic necrotizing lesions and mucosal congestion involved the jejunum and ileum and large intestines, portions of which were blood-filled at necropsy. Lymphoid follicular necrosis was present in the lymph nodes and spleen. The LD_{50} value was found to be 0.376 ± 0.043 mg/kg body weight.

Two female crossbred pigs were administered DON by rapid intravenous infusion at a dose of 0.5 mg/kg body weight; there were two matching controls. Vomiting was observed within a few minutes of dosing, the skin was flushed and the extremities became cold. Pigs had signs of diarrhoea, muscular weakness, tremors, and coma. Symptoms were progressive in severity

reaching a maximum 6–7 h after the injection; recovery occurred after 12 h. Necrosis of pancreatic acinar and islet cells was observed (Coppock et al., 1985). Pigs can ingest up to 2 mg DON/kg feed without suffering any serious toxic effects (Trenholm et al., 1984). T-2 toxin added to the ration at 18 or 30 mg/kg caused the refusal of feed in pigs (Szathmary & Rafai, 1978). Feed refusal and emesis have been produced in other species and by other toxic metabolites of *Fusarium* species (Kotsonis et al., 1975; Vesonder et al., 1977).

The minimum emetic dose of DON in pigs weighing 9–10 kg was 0.05 mg/kg body weight, when administered intraperitoneally, and 0.1–0.2 mg/kg body weight, when given orally. When this toxin was added to feed, the feed consumption of 20–45 kg pigs, was reduced by 20% at a dose of 3.6 mg/kg and by a 90% at a dose of 40 mg/kg (Forsyth et al., 1977). Pigs were about twice as sensitive to DON as rats (Vesonder et al., 1979). DON in contaminated wheat reduced feed intake and weight gain, when it was fed to the pigs. The intake of feed decreased linearly with increasing dietary concentration of DON (Friend et al., 1982). The emetic activity of 15-acetyl DON in pigs was similar to that of DON, the minimum emetic doses being 75 and 50 μg/kg, respectively (Pestka et al., 1987a).

(*b*) *Poultry*

Chi et al. (1977b), reported that the single oral LD_{50} dose of T-2 toxin for one-day-old broiler chicks was 5 mg/kg body weight. It was 5 and 6.3 mg/kg body weight for 8-week-old broiler chicks and laying hens, respectively. Death of the birds occurred within 48 h of T-2 toxin administration. Within 4 h of receiving the toxin, birds developed asthenia, inappetence, diarrhoea, and panting. The abdominal cavities of birds given lethal doses contained a white chalk-like material that covered much of the viscera.

In a study by Wyatt et al.(1972), chickens were fed a diet containing 1–16 mg T-2 toxin/kg feed for 3 weeks. The birds with reduced growth at 4, 8, and 16 mg/kg developed yellow-white lesions in the mouthparts at all dietary concentrations. The lesions consisted of a fibrinous surface layer and a heavy infiltration of the underlying tissues by granular leukocytes.

Escherichia coli and *Staphylococcus epidermis* were isolated from the lesions.

Terao et al.(1978) observed the effects of T-2 toxin and related trichothecenes on the bursa of Fabricius of one-day-old chicks. After injection of 5 mg T-2 toxin/kg body weight into the residual yolk sac, cellular toxic effects were observed on the follicle-associated epithelium, resulting in necrosis, which spread to the periphery. The lesions induced by fusarenon-X and NIV were similar to those induced by T-2 toxin, but the toxins were less potent and their activity was estimated to be more than 40 times less than that of cyclophosphamide.

The acute toxicity of DAS and of T-2 toxin dissolved in dimethylsulfoxide in 7-day-old male broiler chicks was described by Hoerr et al. (1981). The 72-h single oral LD_{50} doses of T-2 toxin and DAS were estimated to be 4 and 5 mg/kg body weight, respectively. Combination of the 2 toxins caused increased mortality in both the single-and multiple-dose tests. Lesions produced by crop gavage with T-2 toxin and DAS were similar, but were more severe in chicks given T-2 toxin. Necrosis of lymphoid tissue and bone marrow was observed in tissue taken 1 h after treatment followed by rapid depletion. Necrosis was observed in the liver, gall bladder, and gut.

Chi et al.(1977c), fed broiler chicks (36 per group), aged one day to 9 weeks, a diet containing T-2 toxin at concentrations of 0.2, 0.4, 2, or 4 mg/kg. Birds fed 4 mg T-2 toxin/kg showed reduced body weight gain and feed consumption and developed oral lesions characterized by circumscribed proliferating yellow caseous plaques at the margin of the beak, the mucosa of the hard palate and the tongue, and the angle of the mouth. No lesions were observed in the bone marrow or, to any significant extent, in the peripheral blood. The no-observed-effect doses of T-2 toxin were 0.2 mg/kg for weight gain, and 0.2 mg/kg for oral lesions. When one-day-old broiler chicks were fed a diet containing 1, 2, 4, 8, or 16 mg T-2 toxin/kg feed for 3 weeks the no-effect doses were estimated as follows: growth rate, weight of pancreas, and weight of spleen: 2 mg/kg; oral lesions: < 1 mg/kg (Wyatt et al., 1973c).

T-2 toxin administered to laying hens at a concentration of 20 mg/kg feed reduced egg production and resulted in the production of a thinner egg shell (Wyatt et al., 1975). Speers et al.(1977), also observed cessation of egg production in hens fed diets containing 25–50 mg monoacetoxyscirpenol/kg or 16 mg T-2 toxin/kg. It was reported by Chi et al. (1977a) that feed consumption, egg production, and shell thickness were significantly decreased in hens fed 8 mg of T-2 toxin/kg. Furthermore, the hatchability of fertile eggs of hens fed 2 or 8 mg T-2 toxin/kg was lower than that of hens fed the control diet (Chi et al., 1977a).

Three groups of 1-day-old chicks (10 chicks per group) were each fed 0.5–15 mg T-2 toxin/kg for 3 weeks (Coffin & Combs, 1981). Plasma-vitamin E activity and hepatic-vitamin A content were measured. Dose-dependent depression of plasma-vitamin E activity was observed, with a 65% decrease compared with controls in chicks fed a diet containing 15 mg T-2 toxin/kg. This decrease was believed to be caused by a reduction in the plasma level of lipoproteins, which are required for the transport of vitamin E.

(*c*) *Ruminants*

In a study by Pier et al. (1976), 4 calves received 0.08–0.6 mg T-2 toxin/kg body weight orally in capsules for 30 days. The high-dose calf developed a hunched stance and died on day 20. At all levels, some evidence of mild enteritis with loose faeces was obtained. Clinically apparent signs were confirmed at doses of 0.16 mg/kg or more, and bloody faeces at doses of 0.32 mg/kg or more. At necropsy, abomasal ulcers were present in the calf given 0.16 mg/kg and ruminal ulcers in calves given the 2 higher doses. Prothrombin times and levels of serum GOT activity were increased in calves given the 2 higher doses.

Ten male Suffolk-Finn-Columbian lambs, in 2 groups of 5 animals each, were fed T-2 toxin at 0.3 or 0.6 mg/kg body weight for 21 days. There were 5 controls. Experimental lambs developed focal hyperaemia and dermatitis at the mucocutaneous junction of the commissure of the lips, diarrhoea, leukopenia, lymphopenia and lymphoid depletion of the mesenteric lymph nodes and spleen (Friend et al., 1983b).

Sheep dosed with roridin A and verrucarin A (4 mg/kg) had severe and extensive haemorrhagic gastroenteritis. Oedema was marked in the abomasum; the small intestine of the roridin-treated lamb had casts of clotted blood and necrotic debris. Both small and large intestines contained grossly haemorrhagic areas and extensive mucosal erosions. In the lamb given verrucarin A at 4 mg/kg body weight, the lesions were sublobular haemorrhages in the liver, which had a nutmeg appearance, mucosal erosions, haemorrhages in the small intestine, and haemorrhages of the endocardium of the left ventricle of the heart (Mortimer et al., 1971).

(d) Cats

Three studies have described the clinical and tissue alterations produced by administration of T-2 toxin to cats (Sato et al., 1975; Lutsky et al., 1978; Lutsky & Mor, 1981). Lutsky et al. (1978) used 20 cats in 4 groups of 4–6 animals each. There were 4 controls. The toxin was administered orally in gelatin capsules on alternative days at doses of 0.06, 0.08, or 0.10 mg/kg body weight, until death. The survival time ranged from 6 to 40 days. The signs included emesis, anorexia, bloody diarrhoea, and ataxia. The cats lost weight and became emaciated. Gross lesions included multiple petechiae to ecchymotic haemorrhages of the intestinal tract, lymph nodes, and heart. The lumen of the gut contained copious amounts of dark red contents. Microscopic lesions included haemorrhages in the gut, lymph nodes, heart, and mininges, necrosis of gastrointestinal epithelium and decreased cellularity of the bone marrow, lymph nodes, and spleen.

(e) Rodents

The trichothecenes used in long-term studies were T-2 toxin and fusarenon-X. Lesions were observed in the oesophageal region of the stomach of DDD mice fed T-2 toxin at 10 or 15 mg/kg diet for 12 months. The alterations included hyperplasia, hyperkeratosis, and acanthosis of the squamous epithelium. Such changes were found 13 weeks after the start of feeding the toxins and were consistently observed during the 12-month feeding period. However, most had subsided 3 months after cessation of feeding. Similar gastric lesions were observed in Wistar rats fed

T-2 toxin at concentrations of 5, 10, or 15 mg/kg feed for 4 weeks. The lesions were diffuse and severe in the rats fed 15 mg/kg, focal but definite in those fed 10 mg/kg, and negligible in the stomach of rats fed 5 mg/kg (Ohtsubo & Saito, 1977).

Six female Holtzman albino rats were fed T-2 toxin at 5 or 15 mg/kg for 19 days and T-2 toxin at 10 mg/kg diet for 8 months. No gastric lesions were observed in any of the animals in the experimental groups (Marasas et al., 1969).

Three groups of 12, six-week-old female Swiss ICR mice (15–20 g body weight) were administered T-2 toxin (by 10-minute aerosol exposure) Two control groups contained 8 mice each using nose-only exposure. The aerosol mass concentration varied between 225 and 275 μg T-2 toxin/litre of air. Tissues from mice were microscopically examined 0.25, 1, 2, 4, 6, 8, 12, and 24 h after exposure. Lymphoid necrosis was observed 1 h after exposure in the thymus, spleen, and lyphoid nodules of the intestinal tract. Necrosis of intestinal crypt epithelial cells was present 2 h after exposure and necrosis of adrenal cortical cells 4 h after exposure (Thurman et al., 1988).

In male Sprague-Dawley rats, T-2 toxin, given intravenously, produced reduced blood flow and increased vascular resistance in hind-quarter, mesenteric, and renal vascular beds. Mean arterial pressure and heart rate were not significantly altered. A maximum drop in blood flow in mesenteric and renal vascular beds occurred 4 h after the T-2 toxin was injected (Siren and Feuerstein, 1986).

II.4.2.2 Haematological and haemostatic changes

A haemorrhagic syndrome was reported to be the characteristic feature of mouldy corn toxicosis in the cow (section II.4.1.). However, this hemorrhagic syndrome could not be produced in other studies.

In a study by Patterson et al. (1979), 2 calves were administered 0.2 mg T-2 toxin/kg body weight and one calf was given the same dose of DAS; both compounds were given by stomach tube,daily for 11 days. There were no controls. The T-2-treated animals developed clinical signs of weakness, inappetence, and one died.

Prothrombin time was prolonged in both animals and one had marked neutrophilia. No clinical signs or haematological changes were observed in the animal administered DAS. No haemorrhagic syndrome was found in these calves.

When pigs (9–10 weeks old, male, castrated, specific pathogen-free) were exposed to a T-2 toxin aerosol (390 μg/litre, 15 μm mass median aerodynamic diameter) for a period that allowed an amount equivalent to 8 mg/kg to be nebulized, the haematological alterations included a decrease in lymphocyte and neutrophil counts, and decreased concentrations of serum-protein and haemoglobin (Pang et al., 1988).

Haematological changes were observed in mice, rats, cats, and guinea-pigs treated with T-2 toxin and related trichothecenes (Sato et al., 1975, 1978; Sato & Ueno, 1977; DeNicola et al., 1978). In cats, leukocytosis occurred early after the administration of T-2 toxin. A similar change was observed in mice treated with T-2 toxin, neosolaniol, and fusarenon-X. Among the leukocytes, lymphocytes showed the greatest increase followed by neutrophils; the leukocytosis was followed by marked leukopenia after short-term exposure to T-2 toxin. This leucopenic state was also induced by DAS in mice (Conner et al., 1986), rats and dogs (Stahelin et al., 1968) and by verrucarin A in rats, dogs, guinea-pigs, and monkeys (Rusch & Stahelin, 1965). Pancytopenia was also reported in cats administered T-2 toxin for 2 weeks (Lutsky et al., 1978; Lutsky & Mor, 1981). In guinea-pigs treated with T-2 toxin (0.9 mg/kg body weight per day) for 27 days, erythropenia, leukopenia, and absolute lymphopenia were observed, with a marked decrease in the lymphocyte contents of the bone marrow (DeNicola et al., 1978).

Hayes et al. (1980) studied the effects of T-2 toxin on the haematopoiesis in mice. Twenty-four male weanling outbred Swiss mice were fed a balanced semipurified diet, containing crystalline purified T-2 toxin at a level of 20 mg/kg dry diet. One group of 20 animals received the toxin in the diet for 41 days and another group of 4 animals, for 21 days, followed by control diet for 7 days. Forty-eight animals in 3 groups served as controls and received the semipurified diet with restricted intake (20 animals for 41 days), and ad lib (8 animals for 28 days). In

addition, 12 animals were sacrificed at day 0. Haematological studies were made at weekly intervals. During the first 3 weeks of exposure to T-2 toxin, lymphoid tissues, bone marrow, and splenic red pulp became hypoplastic resulting in anaemia, lymphopenia, and eosinopenia. Subsequently, during continued exposure to T-2 toxin, there was regeneration leading to hyperplasia of the haematopoietic cells by 6 weeks. All animals also developed perioral dermatitis and ulceration of the gastric mucosa. The above results indicate both the irritant and haematopoietic suppressive effects of the T-2 toxin. However, the haematopoietic effects were transient at the dose administered and did not lead to haematopoietic failure.

The haemostatic derangements produced by T-2 toxin have been studied in the guinea-pig (Cosgriff et al., 1984), rabbit (Gentry, 1982), chicken (Doerr et al., 1981), and monkey (Cosgriff et al., 1986). Guinea-pigs (Hartley strain, number not stated) administered T-2 toxin dissolved in ethanol, by intramuscular injection, at a dose of 1 mg/kg body weight (LD_{50}-24 h) developed decreased activities of all coagulation factors except fibrinogen. Platelet aggregation in whole blood response to ADP and collagen was depressed. The animals also showed an initial rise followed by a fall in the haematocrit level, leukocytosis, and a fall in platelet count. These changes, which were found within a few hours of toxin administration, reached a maximal at 24 h and returned to normal over the next 2 days. Pretreatment with vitamin K_1 did not prevent the effects of T-2 toxin on coagulation. The addition of T-2 toxin to the plasma and blood of untreated guinea-pigs at a concentration of 1 mg/litre did not have any effect on clotting times or platelet aggregation, indicating that the T-2 toxin itself did not have any direct effect on the activity of coagulation factors (Cosgriff et al., 1984).

Eight New Zealand White rabbits were administered T-2 toxin dissolved in dimethyl sulfoxide (DMSO) by intravenous injection at 0.5 mg/kg body weight; 5 rabbits were given a single oral dose of 2.0 mg/kg body weight. In the rabbits treated intravenously, both the packed cell volume and the total leukocyte counts were reduced. However, no significant alterations occurred in the haematological parameters of rabbits given the T-2 toxin orally (Gentry & Cooper, 1981).

In another study, 9 New Zealand White rabbits were given a single intravenous injection of T-2 toxin dissolved in DMSO at a dose of 0.5 mg/kg body weight. A second group of 5 animals received daily subcutaneous injections of vitamin K, at a dose of 0.5 mg/kg body weight, for 5 days prior to administration of a similar dose of T-2 toxin and for a subsequent 4 days. A total of 16 rabbits in 2 groups served as controls. Blood samples were examined from each animal before toxin or DMSO treatment and 6–96 h later. Several coagulation factors (VII, VIII, IX, X, XI) were decreased by about 40% within 6 h of toxin administration in the group administered toxin alone. Fibrinogen content was elevated at 24 h. However, the reduction in the coagulation factors did not induce clinical haemorrhage and administration of vitamin K did not alter the effects of T-2 toxin administration, indicating that the mechanism of action of the toxin on coagulation was not as a vitamin K antagonist.

In a study by Cosgriff et al. (1986), 9 Cynomolgus monkeys received an intramuscular injection of an LD_{20} dose (0.65 mg/kg body weight) of T-2 toxin dissolved in ethanol. Three monkeys served as controls. Haematological studies were made before toxin injection and at different intervals from 6 to 24 h and 2 to 7 days after treatment. The animals were studied for signs of toxicity and particularly for evidence of haemorrhage. Necropsy was performed on animals that died during study. Leukocytosis levels in treated animals were 4–5 times pretreatment levels. Prolongation of prothrombin, activated thromboplastin times, and a decrease in multiple coagulation factors were also observed. These changes were detected within hours of toxin administration, reached a maximum at 24 h, and returned to normal over the next 3 days. Fibrin-fibrinogen degradation products were not detected at any time. Platelet counts which were unchanged in treated animals, were significantly raised in control animals following repeated phlebotomies. None of the animals developed the haemorrhagic syndrome. Five animals that died during the study showed mild peticheal haemorrhages involving the colon and heart, as well as necrosis of lymphoid tissues.

Rukmini et al. (1980) conducted a study on adult rhesus monkeys in which 3 males and 2 females were administered pure T-2 toxin

in 20 ml milk by stomach tube, daily, initially at 1 mg/kg body weight for 4 days, and then at 0.5 mg/kg body weight from day 5 to day 15. Three males and 3 females served as controls. All 3 males in the treated group died of respiratory failure between days 0 and 15. Subsequently, after 30 days recovery, the 2 treated female and 2 additional male monkeys received 0.1 mg T-2 toxin/kg body weight for 15 days. All monkeys given 1 mg T-2 toxin/kg per day showed signs of toxicity similar to those of alimentary toxic aleukia in man, i.e., vomiting, apathy, and weakness of lower limbs. The signs were more severe in males, and they also developed peticheal haemorrhage on the face. All male animals developed severe leukocytopenia, follicular atrophy of the spleen and lymph nodes, and pneumonia, suggesting involvement of the immune system. At a dose of 0.1 mg/kg per day, both male and female animals developed leukocytopenia and mild anaemia after 15 days of treatment.

The effects of T-2 toxicosis on blood coagulation were studied in groups of 40, day-old chickens fed diets containing the toxin at concentrations of 1, 2, 4, 8, or 16 mg/kg. Forty animals served as controls. Factor X, and prothrombin and fibrinogen activities were reduced only at the highest dietary dose, whereas Factor VII was reduced at dietary doses of 4, 8, and 16 mg/kg and was the most sensitive of the clotting components to T-2 toxin toxicosis. Thus, T-2 toxin toxicosis induced by high doses results in multiple-factor coagulopathy and mild toxicosis results in a deficiency of Factor VII (Doerr et al., 1981).

II.4.2.3 Disturbances of the central nervous system

Four-week-old male broiler chickens were intubated with a single dose of T-2 toxin at 2.5 mg/kg body weight, and the brain concentrations of dopamine, norepinephrine, and serotonin, and selected blood components were determined 4–48 h after administration. There was a significant elevation in the brain-dopamine concentration and a reduction in the brain-norepinephrine concentration. The brain-serotonin contents did not change (Chi et al., 1981). Batches of 40 broiler chickens fed graded concentrations of 1–16 mg T-2 toxin/kg feed for 3 weeks developed an abnormal positioning of the wings,

hysteroid seizures, and impaired righting reflex. Neural toxicity, which occurred at levels above 4 mg T-2 toxin/kg diet, might have been related to alterations in brain biogenic amines (Wyatt et al., 1973a,b; Chi et al., 1977a).

Signs of nervous system dysfunction (restlessness, dyspnoea, ataxia) were observed in rats after subcutaneous or intracerebral injection of T-2 toxin (10–20 μg toxin) or after intracerebral implantation of toxin adsorbed on talc (Bergmann et al., 1985). Weanling, male Wistar rats were administered T-2 toxin orally at 2.0 mg/kg body weight and the concentrations of neurotransmitters determined. The toxin increased concentrations of tryptophan, serotonin, and dopamine in the brain, but decreased concentrations of 3,4-dihydroxyphenylacetic acid (MacDonald et al., 1988). Male Sprague-Dawley rats (180 g) were dosed orally with DON or T-2 toxin at 21.5 mg/kg body weight. Both the toxins significantly increased serotonin and 5-hydroxy-3-indoleacetic acid concentrations in all regions of the brain examined, whereas norepinephrine and dopamine concentrations were not altered (Fitzpatrick et al., 1988). Male Sprague-Dawley rats received 1 mg T-2 toxin/kg body weight by intravenous injection. Concentrations of vasopressin, oxytocin, and leucine enkephalin decreased in the posterior pituitary and concentrations of methionine enkephalin increased (Zamir et al., 1985).

Male Sprague-Dawley rats (180 g) and 4-week-old White Leghorn cockerels were dosed orally with DON at 2.5 mg/kg body weight. Whole brain concentrations of monoamine neurotransmitters were not altered in either species. The treatment produced elevated concentrations of serotonin and 5-hydroxy-3-indoleacetic acid in the rat, but not in the chicken (Fitzpatrick et al., 1988).

II.4.2.4 Dermal toxicity

After the discovery of the skin-necrotizing property of toxic metabolites of *F. sporotrichioides* and related fungi (Joffe, 1962), the skin irritation test was introduced for the screening of toxins and metabolites of *Fusarium* species (see section II.1.2.3). T-2 toxin, HT-2 toxin, and DAS were isolated from cultures of

F. tricinctum using the skin test for selection of active fractions (Gilgan et al., 1966; Bamburg et al., 1968b). Toxins, such as T-2 toxin, HT-2 toxin, and DAS are extremely potent irritants while NIV and fusarenon-X are much less so (Bamburg et al., 1968b; Ueno et al., 1970; Wei et al., 1972; Chung et al., 1974; Hayes & Schiefer, 1979; Bhavanishankar et al., 1988).

The mechanism of the skin toxicity of trichothecenes has not been established. Results of studies with fusarenon-X have indicated that the vascular permeability of the skin of the back of the rabbit, estimated by exudation of a vital dye (pontamine sky blue), was biphasic reaching maxima 5 and 24 h after topical application. These data indicate that increased vascular permeability is one of the early responses of the skin to these toxins and that some chemical mediators participate in the biphasic increase in vascular permeability (Ueno, 1980a).

II.4.2.5 Impairment of immune response

Experimental animal studies show that some trichothecenes affect the immune system and thereby modify the immune response. The impairment comprises the following functions: antibody formation; allograft rejection; delayed hypersensitivity; and blastogenic response to lectins. As a consequence of the impairment, decreased resistance to microbial infection has been experimentally established. It is likely that the impairment of the immune system is linked to the inhibitory effect of trichothecenes on macromolecule synthesis.

(*a*) *Antibody formation*

Rosenstein et al.(1979) showed that T-2 toxin and DAS inhibited responsiveness to sheep red blood cells in male Swiss IC and C5781/6 mice. In their first study, T-2 toxin or DAS was injected ip daily for 7 days at 0.75 mg/kg body weight, in 6 groups of 4 mice each, with matching controls. Mice were immunized with sheep erythrocytes (SRBC) on day 3 after treatment and killed 5 days after immunization. Both toxins produced a fall in anti-SRBC titres measured by haemagglutination and reduced thymic weight. In a second study, 7 groups of 5 mice each were administered daily (ip) doses of T-2 toxin ranging from 0 (solvent

alone) to 2.5 mg/kg body weight over a 7-day period. The same number of mice received DAS under similar conditions. Mice were immunized on day 3 and killed 5 days later. Antibody-producing cells from the spleen were counted by numbering the plaque-forming cells (PFC) on sheep erythrocytes. A dose-dependent inhibition of PFC was observed in T-2 toxin-treated mice, with a total suppression of the immune response at 2.5 mg/kg. The effects of DAS were less. A subsequent follow-up of the evolution of the immune response in 36 mice administered T-2 toxin (ip) at 0.75 mg/kg body weight daily for 7 days indicated that the immunosuppressive effect disappeared within 6 days followup cessation treatment.

The T-cell-independent-responses-production of anti-polyvinyl-pyrrolidone and anti-dinitrophenol-ficoll-antibodies were enhanced by T-2 toxin and DAS. T-2 toxin-treated mice produced 50% fewer plaque-forming cells against SRBC. There was also a decreased response to phytohaemagglutinin in splenic cells from treated mice. However, Masuko et al. (1977), and Otokawa et al. (1979), reported that a single dose of 3 mg T-2 toxin/kg body weight in mice caused modification of delayed hypersensitivity responses without affecting antibody response. This apparent contradiction was explained by a difference in the timing of the administration of T-2 toxin, mice receiving the toxin once, several days before or after antigen-stimulation. Results of studies with fusarenon-X indicated that both IgE and IgG antibody responses to DNA-OVA were suppressed when male BALB/c mice were repeatedly dosed with mycotoxin at doses exceeding 25 mg/day; the inhibition of antibody-formation was greater when given 7 days before antigen-stimulation. In mice stimulated with pokeweed mitogen and lipopolysaccharides, the *in vitro* antibody production by splenic cells from fusarenon-X-treated mice was suppressed (Masuda et al., 1982).

Sato et al.(1981), examined the effects of fusarenon-X on serological responses in chicks inoculated with Newcastle disease vaccines. When chicks were fed 8 mg fusarenon-X/kg for 6 weeks, no significant reductions in body or organ weights were observed. However, haemagglutinin inhibitory antibody titres were reduced when chicks were immunized with live, but not with inactivated, vaccine.

Mann et al. (1983), reported alterations in the levels of several serum proteins in calves orally administered T-2 toxin (0.6 mg/kg per day over 43 days). Total protein, albumin, and immunoglobulin fractions were decreased in toxin-treated calves, including the α-β_1-and β_2-globulin fractions. IgA and IgM values and complement proteins were lower in treated calves.

Sublethal doses of DON (0.25, 0.50, or 1.0 mg/kg feed) were fed for 54 weeks, beginning at 21 days of age, to a total of 96 weanling male Swiss Webster mice divided into 4 groups. There were 32 controls. The dose of 1.0 mg/kg reduced serum α_1- and α_2-globulins, increased serum-albumin levels, and reduced feed consumption and body weight gain. The dose of 0.5 mg/kg reduced α_2- and β-globulins (Tryphonas et al., 1986).

T-2 toxin was fed to 6 weaned pigs at 5 mg/kg feed for 25 days and the immune response evaluated by *in vitro* testing for blast transformation, immune-rosette formation, and IF-detectable IgG-positive cell counts. T-2 toxin produced a 40–50% reduction in immune responsiveness and a decrease in total leukocyte count, but an increase in adrenocortical activity. Neutralizing antibody titres to vaccination with enteritic B vaccine were lower in the treated pigs. It was concluded that T-2 toxin had a distinct immunosuppressive effect during the early phase of immune induction by altering the function of both T- and B-lymphocytes (Rafai & Tuboly, 1982).

Dietary DON (2, 5, or 25 mg/kg feed for 2 or 8 weeks) depressed the plaque-forming response to sheep erythrocytes in splenic cultures from $B6C3F_1$ mice. Some effect on the plaque-forming response was detectable with both the 2- and the 8-week period of feeding (Pestka et al., 1987b). DON, given by gavage at 0.75, 2.5, or 7.5 mg/kg body weight also reduced serum-IgM response to sheep erythrocytes and plaque-forming cell numbers were lower in the treated groups (Tryphonas et al., 1984).

(*b*) *Allograft rejection*

Observations on the inhibition of cellular immunity by trichothecenes have included responses to grafting. According to Rosenstein et al.(1979), the mean survival time of the skin grafted from C57Bl/6 mice on to Swiss mice was 8.69 days in the control

recipients. However, when the recipients were treated with 0.75 mg T-2 toxin/kg per day for 7 days before skin graft and then 3 times a week for 20 days, the mean survival time of the graft was increased to 12 days, indicating that T-2 toxin suppressed certain steps of immunity resulting in allograft rejection. The areas of the graft in T-2 toxin-treated mice lacked the typical cellular infiltrates of a cell-mediated immune response of macrophages and lymphocytes.

(*c*) *Delayed hypersensitivity*

Delayed hypersensitivity (DH) is an immune response mediated by sensitized T lymphocytes. The possible impairment of T lymphocytes by T-2 toxin was studied in female BDF_1 mice sensitized by the sc injection of sheep erythrocytes (SRBC) followed by estimation of foot pad swelling. When mice received 3 mg T-2 toxin/kg body weight, before, or on the day of, sensitization, no appreciable effect on DH was observed. However, when the toxin was administered 2 or 3 days after sensitization, marked enhancement of the delayed hypersensitivity response was seen (Otokawa et al., 1979). This indicates that the timing of toxin exposure was critical for enhancement of the delayed hypersensitivity response. Since the life-time of the effective T-2 toxin dose *in vivo* was very short, and the optimal timing of toxin injection corresponded with the time of appearance of suppresser cells, it was presumed that trichothecenes might interfere with the proliferation of suppresser T cells that appear in DH-tolerant mice.

DON fed to $B6C3F_1$ mice at 2, 5, or 25 mg/kg for 2 or 8 weeks depressed the delayed hypersensitivity response to keyhole limpet haemocyanin. The effects on hypersensitivity were detectable in mice fed the mycotoxin for 2 weeks, but disappeared when the feeding period was extended to 8 weeks (Pestka et al., 1987b).

(*d*) *Blastogenic response to lectins*

Certain mitogens, such as phytohaemagglutinin (PHA) and concanavalin A, stimulate the proliferation of T cells *in vitro*; lipopolysaccharide (LPS) causes the same phenomena in B cells. Lafarge-Frayssinet et al. (1979) investigated the responses of lymphocytes to PHA and LPS in mice treated with crude T-2

toxin and DAS. Mice were treated with the mycotoxins at doses of one-quarter of the LD_{50} or one-twelfth of the LD_{50} for 15 days and the response of splenic or thymic cells to the mitogens was examined. The data indicated that stimulation of both T and B cells was inhibited reversibly, and that the ability to synthesize anti-SRBC antibodies was suppressed. *In vitro* effects on lymphocytes and fibrosarcoma cell cultures included a direct cytostatic action at high concentrations and a stimulating action at low concentrations. Histopathological observations included severe lymphoid damage in the thymus and spleen. The results of these studies indicate that the immune system appears sensitive to the trichothecenes and is impaired at doses not inhibitory for other organs.

Swiss mice were fed a diet containing T-2 toxin at 5, 10, or 20 mg/kg for 1, 2, 3, 4, or 6 weeks. The ingestion of T-2 toxin (only at 20 mg/kg at 3 weeks) depressed total splenic cell counts. T-2 toxin at 20 mg/kg for 1–4 weeks decreased splenic proliferative responses to T-cell mitogen concanavalin A; however, the response to a lipopolysaccharide (LPS), B-cell mitogen, was decreased in mice fed T-2 toxin at 10 or 20 mg/kg for 1–4 weeks. (Friend et al., 1983a).

Lymphocytes from calves exposed to T-2 toxin at 0.6 mg/kg for as long as 43 days had a decreased response to the mitogen, PHA, on days 1, 8, and 29 after toxin administration. Lymphocyte responses to concanavalin A and pokeweed mitogen were also observed on day 29 after dosing (Buening et al., 1982).

In a study to determine the effects of T-2 toxin on the bovine immune system, calves (5) were orally dosed with 0.3 mg/kg per day, for 56 days. Neutrophil function was reduced by treatment with T-2 toxin as was the cutaneous reaction to injected phytohaemagglutinin. In a second study, calves (6) were given T-2 toxin at a dose of 0.5 mg/kg per day, for 28 days. B-lymphocyte number and the response of the B-cell-enriched fraction to phytohaemagglutinin both increased after treatment. The *in vitro* exposure of mononuclear cells, B-cell-enriched or T-cell enriched fraction, reduced the lymphoblastic response to mitogens. A 50% reduction was induced by 1.4 ng T-2 toxin/ml (Mann et al., 1984).

The effects of T-2 toxin on *in vitro* mitogen response and antibody production by human peripheral blood lymphocytes were reported by Tomar et al. (1988). The toxin inhibited the mitogen response to concanavalin A at a lower concentration (1.6 mg/ml) compared with phytohaemagglutinin (2–4 mg/ml) and pokeweed mitogen. In the presence of the toxin, inhibition reached a maximum during first 8 h. The results indicate that various subpopulations of lymphocytes have different susceptibilities to T-2 toxin.

Mitogen-induced blastogenesis in cultured human lymphocytes was inhibited by T-2 toxin and its metabolites. The concentrations of T-2 toxin, HT-2, 3′-OH T-2,3′-OH HT-2, T-2 triol, and T-2 tetraol toxins that produced 50% inhibition of ^{3}H-thymidine uptake in mitogen-stimulated human peripheral lymphocytes were 1.5, 3.5, 4.0, 50.0, 150.0, and 150.0 ng/ml, respectively. The initial hydrolysis of T-2 toxin to HT-2 toxin and the hydroxylation to 3′-OH T-2 did not significantly decrease the immunotoxicity (Forsell et al., 1985). Other trichothecenes were less toxic than T-2 toxin in this system. The doses that produced 50% inhibition of ^{3}H-thymidine uptake in mitogen-stimulated human lymphocytes for fusarenon-X, NIV, DON, and 15-AcDON were 18, 72, 140, and 240 ng/ml, respectively. These results indicate that the lymphotoxicity of trichothecenes is related to the C-4 substituent (Forsell & Pestka, 1985).

T-2 toxin was examined for its effects on lymphocyte activation and interleukin-2 production by splenic cultures from mice. Splenic cells were taken from female BALB/c mice given 2 mg T-2 toxin/kg body weight by stomach tube for 4 days or 4 mg T-2 toxin/kg by stomach tube in single dose. Cells were incubated with 1 μg concanavalin A and the synthesis of cellular protein and DNA determined. The single dose of 4 mg/kg did not alter lymphocyte activation, but the dose of 2 mg/kg for 4 days produced a 50% reduction in activation. The supernatant from these cells had 4 times greater interleukin-2 activity (Holt et al., 1988a).

DON and 3-AcDON were evaluated *in vitro* for their effects on mitogen-induced lymphocytic blastogenesis using rat or human peripheral blood lymphocytes. Both mycotoxins produced a

dose-dependent reduction in lymphocytic proliferation and DON produced a greater inhibitory effect than the acetylated compound. Thus, the concentrations of DON producing 50% inhibition of blastogenesis were 90 and 220 ng/ml for rat and human lymphocytes, respectively. The values for 3-AcDON were 450 and 1060 ng/ml, respectively (Atkinson & Miller 1984).

(*e*) *Resistance to infection*

The immunosuppressive effect of trichothecenes has resulted in an increased incidence and severity of infection in animals in several studies. According to Boonchuvit et al. (1975), an increased mortality rate was recorded when 40 chickens were fed a diet containing 16 mg T-2 toxin/kg for 1 week and were then inoculated orally with 1×10^8 cells of *Salmonella*.

The depression of resistance to experimental tubeculosis by T-2 toxin was studied in mice by Kanai & Kondo, (1984). Groups of male mice, strain ddY, 18–20 g body weight, with 10–14 animals per group, were administered mycobacteria by intravenous injection in the tail vein. In the first study, the doses injected consisted of 0.01 mg culture of tubercle bacteria per animal (species not indicated). One group was administered 0.1 mg T-2 toxin per animal a total of 12 times orally, starting on the day before the injection, 7 times with one-day intervals, and then 5 times daily. For comparison, a second group of mice was administered 5 mg cortisone acetate per animal ip under a similar time schedule. A third group was injected with tubercle control bacteria only. At the end of the 20-day observation period, the mice in the first group had a lower spleen weight and a higher tubercle bacteria count in the spleen than those in the other 2 groups, indicating a more pronounced depression of resistance by T-2 toxin than by cortisone. In a second study, two groups of animals were injected with 0.25 mg of a culture of *Mycobacterium bovis*; one group was then administered 0.1 mg T-2 toxin per animal daily for 6 days, starting 8 days after injection. There were two groups of controls. The average survival time in the T-2 toxin-treated group was reduced to 19 days, compared with 35 days in the untreated group, indicating decreased resistance.

Rats were injected ip with T-2 toxin in a single dose of 1 mg/kg body weight or in doses of 0.5 mg/kg daily for 5 days. The rats

were then inoculated with 0.1 ml of medium containing 10^9 *Staphylococcus aureus*/ml. Rats given multiple intramuscular injections of T-2 toxin showed more oedema and myofibre necrosis at the injection site of the bacteria; the cellular infiltrate was sparse and bacteria were abundant. Bone marrow myeloid cells were markedly decreased by multiple injections of T-2 toxin. In *in vitro* studies, small, non-lethal doses of T-2 toxin inhibited the chemotaxis of leukocytes and decreased phagocytosis of the bacteria by leukocytes (Yarom et al., 1984b).

Seven male rhesus monkeys (*Macaca mulatta*) were dosed daily by stomach tube for 4–5 weeks with 100 μg T-2 toxin/kg body weight. This dose resulted in the death of 3 animals, 40% reduction in leukocyte counts, reduction in the bactericidal activity of neutrophils (phagocytosis of *E. coli*), reduction in the transformation of lymphocytes by mitogens, and a reduction in numbers of C-cell and T-cell lymphocytes (Jagadeesan et al., 1982).

The immunotoxic effects of T-2 toxin on cell-mediated resistance were studied in female ICR mice infected with *Listeria monocytogenes*. Mice in groups of 17 animals (10 animals in the control group) were inoculated ip with 4×10^5 (LD_{50}) or 4×10^4 (non-lethal) doses of *L. monocytogenes* per animal, treated with a single oral dose of 4 mg T-2 toxin/kg body weight, and observed for 15 days. Bacterial multiplication was rapid in the spleen after T-2 toxin treatment and mortality was increased in both treated groups. Necrosis and depletion of lymphoid tissue were observed in the thymus, the periarterioler lymphoid sheaths and the lymphoid follicles of the spleen. Cellular response to *L. monocytogenes* in the spleen and liver was decreased by treatment with T-2 toxin and the lesions were sparsely populated with mononuclear cells. The foci of necrosis were larger with numerous colonies of bacteria. The influx and number of lymphocytes and macrophages were greater in *Listeria*-elicited peritoneal exudates. The immunotoxic effects of T-2 toxin were comparable with those produced by cyclophosphamide and were attributed to depletion of T lymphocytes and subsequent failure of T-cell-dependent macrophages to clear the host of bacteria (Corrier & Ziprin, 1986a). In a continuation of the previous study, female ICR mice (17 animals per group) were inoculated ip on day 1 with 4×10^5 (LD_{50}) or 4×10^4 (non-lethal) bacteria

per animal, treated orally on day 0, 1, 2, and 3 with 0, 1, or 2 mg T-2 toxin/kg, and observed for 15 days. The suppression of resistance by the mycotoxin was indicated by rapid multiplication of *Listeria* in the spleen and increased mortality in mice in both exposed groups treated with 2 mg/kg. The thymuses and spleens of toxin-treated mice showed necrosis and depletion of lymphoid cells. Foci of necrosis induced by *Listeria* infection in the spleen and liver were larger in treated mice and the inflammatory reaction was sparse (Corrier & Ziprin, 1986a,b).

Increased resistance to *L. monocytogenes* infection was surprisingly observed by the same group (Corrier & Ziprin, 1986b) in mice administered T-2 toxin several days prior to the inoculation with bacteria. Female ICR mice, 16 animals per group, were administered T-2 toxin by stomach tube at dosage levels of 2, 1, 0.5, or 0 mg toxin/kg body weight, on days –5, –4, –3, –2, –1, +1 and +3. On day 0 the two treated groups were inoculated ip with 10^6 (LD_{100}) and 10^5 (LD_{50}) *L. monocytogenes,* respect-ively. In addition, 20 mice were given 2 mg T-2 toxin/kg on the same days as above, and used to determine the effect of the toxin. Although the cytotoxic effect of T-2 toxin on lymphoid tissue was marked, enhanced resistance to *Listeria* infection was revealed by a decrease in mortality due to listeriosis (in both bacteria-exposed groups) in a T-2 toxin dose-dependent way. No specific cause for the increased resistance to listeriosis by T-2 toxin treatment prior to bacterial infection was identified by the authors.

ICR female mice were treated with the trichothecene mycotoxin DAS and subsequently inoculated ip with *Listeria monocytogenes.* The effect of the mycotoxin on the course of the infection was monitored by observing the resultant mortality and the bacterial content of the spleens from inoculated mice. Mice given 3 mg DAS/kg body weight orally, on 2 and 1 days before inoculation, showed increased mortality and splenic *Listeria* counts. In these mice, thymus weights were reduced, and lymphocytes were depleted from the thymus cortex and from splenic lymphoid follicles and periarteriolar lymphoid sheaths. A single dose of 4 mg DAS/kg given on day 6 before challenge exposure did not affect mortality compared with controls. Mice treated with DAS and subsequently inoculated with *Listeria* had significantly ($P = 0.006$) higher levels of neutrophil

populations than *Listeria*-infected control mice (Ziprin & Corrier, 1987).

Dietary DON decreased resistance of B6C3F1 mice to infection with *L. monocytogenes*. Resistance to the infection was simi-larly decreased in control mice fed restricted diets, comparable to dietary restriction caused by DON-induced feed refusal. Resistance to *L. monocytogenes* was reduced to a greater extent by feed containing both DON and zearalenone (Pestka et al., 1987b). DON in the diet at 0.50 or 1.0 mg/kg was fed for 5 weeks. Both doses resulted in a dose-related decrease in time-to-death interval following challenge with *L. monocytogenes* (Tryphonas et al., 1986).

T-2 toxin was fed to young male white Swiss mice at doses of 10 or 20 mg/kg diet for 2–3 weeks. The mice were then inoculated ip with herpes simplex virus (HSV-1). Mice fed the high dose of T-2 toxin were highly susceptible to HSV-1 infection and about 75% died with extensive hepatic and adrenal gland necrosis and with little or no inflammatory cellular reaction in affected tissues, such as the liver and adrenal glands, and the central nervous system. No necrotizing encephalitis was found in treated mice. Mice fed 10 mg T-2 toxin/kg had lesions of intermediate severity between those of the high-dose group and the virus-infected controls (Friend et al., 1983c). Feeding of T-2 toxin at 5, 10, or 20 mg/kg for 3–6 weeks did not reactivate the virus in mice latently infected with HSV-1 (Friend et al., 1983a).

Mice (male, Swiss strain weighing 25 g) received inoculations (route not stated) of *Cryptococcus neoformans* (1×10^6 cells) and ip doses (1/8 or 1/4 LD_{50}) of DAS on days 5, 6, and 7 after inoculation with the fungal cells. No deaths occurred in either the *C. neofomans*-treated groups or the T-2-toxin group. A marked additive effect on mortality was observed when mice received both *C. neoformans* and T-2 toxin (Fromentin et al., 1981).

II.4.2.6 Carcinogenicity

The IARC (1983) studied the experimental data on the carcinogenicity of T-2 toxin and concluded that no evaluation of the

carcinogenic role of T-2 trichothecene in experimental animals could be made, because of the inadequacy of experimental data.

In a 16-month feeding study, groups of 50 male and 50 female weanling CD-1 mice were fed a semi-synthetic diet containing 1.5 or 3.0 mg T-2 toxin/kg. Survival was lowest in the control group. No statistically recognizable differences were found in feed consumption or body weight gains among the groups. Statistically significant differences were found in the incidence of pulmonary adenomas and hepatic adenomas in the males of the 3.0 mg/kg group and the controls. Other treatment-related findings were an increased prevalence of epithelial cell hyperplasia and hyperkeratosis in the stomach of animals fed the T-2 toxin diets (Schiefer et al., 1987).

In an attempt to determine the carcinogenicity of NIV, a study was conducted in which mice were fed mouldy rice for 2 years. Groups of 42, 7-week-old female C57BL/6CrSlc SPF mice were fed diets containing 6, 12, or 30 mg NIV/kg for 2 years, and were assessed for the effects on body weight gain, feed efficiency, terminal organ weights, haematological values, and lesions. The mortality was lowest in the highest dose group, followed by the 12 mg/kg group; body weight gains and feed efficiency were dose-dependently reduced. No particular neoplasms attributable to treatment were found. The incidence of naturally occurring neoplasms, mostly lymphomas, was similar in all groups. On gross and microscopic examination of the liver, thymus, spleen, kidneys, stomach, small intestines with or without Peyer's patches, no alteration related to treatment was observed except for amyloidosis, which was lower in the two higher dose groups (Ohtsubo et al., in press).

Two groups of 16 or 18 DDD male mice received either 10 or 20 weekly sc injections of 2.5 mg fusarenon-X/kg body weight. No increase in tumour incidence was noted in treated animals, compared with the controls (Saito & Ohtsubo, 1974).

In a feeding study, fusarenon-X, at a dose of 3.5 or 7 mg/kg diet was fed to 151 male Donryu rats for 1–2 years. Treatment with the mycotoxin reduced the growth rate, but did not induce any carcinogenic effects (Saito et al., 1980).

In studies to investigate skin tumour induction using T-2 toxin and DAS, the skin of the back of mice was painted with 0.1–1 mg of trichothecenes, twice a week, for one year. A notable finding was necrosis of the skin, but no tumours were detected. The skin tumour induction test was also carried out using the initiator-promotor procedure. T-2 toxin and fusarenon-X were not promoting agents of dimethylbenz(*a*)anthracene-induced skin neoplasia. According to Lindenfelser et al. (1974), neither T-2 toxin nor DAS served as initiating agents.

II.4.2.7 Mutagenicity

In a Rec-assay using *Bacillus subtilis*, DNA damage was not induced by 20 or 100 μg of eitherT-2 toxin or fusarenon-X (Ueno & Kubota, 1976). Such trichothecenes as T-2 toxin, DAS, and DON were not mutagenic to *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538, with or without S-9 fraction from rat liver (Kuczuk et al., 1978; Ueno et al., 1978; Wehner et al., 1978a).

T-2 toxin and DAS were not mutagenic in the D-3 mitotic recombination test with *Saccharomyces cerevisiae* (Kuczuk et al., 1978).

Neither DNA-strand breakage nor induction of 8-azaguanine-resistant mutation were detected with 1–32 mg fusarenon-X/litre in Hela cells and with the same toxin at 0.1–1.0 mg/litre in FM3A cells derived from C3H mouse mammary carcinoma cell line (Umeda et al., 1972, 1977). On the other hand, Lafarge-Frayssinet et al. (1981) reported that T-2 toxin induced single-strand breaks in the DNA of lymphoid cells *in vivo* (3 mg/kg body weight) and *in vitro* (0.7–5 ng/ml). Such DNA breakage was not observed in hepatic cells.

T-2 toxin, NIV, and fusarenon-X produced weak clastogenic effects in Chinese hamster V79-E cells (Thust et al., 1983). Both T-2 toxin and HT-2 toxin inhibited the incorporation of tritiated thymidine into the DNA of human fibroblasts in culture in a dose-related fashion. Non-toxic and toxic doses of DON (0.1–1000 mg/litre), did not significantly increase unscheduled DNA synthesis in primary cultures of rat hepatocytes (Bradlaw et al., 1985).

Norppa et al. (1980) also reported weak induction of chromosomal aberrations by T-2 toxin (1.7, 2.7, or 3.0 mg/kg body weight) in Chinese hamster bone marrow cells. The bone marrow micronucleus test was negative at a dose of 3 mg T-2 toxin/kg body weight resulting in a significant decrease in polychromatic erythrocytes.

Hamsters fed T-2 toxin for 6 weeks (2.5 mg/kg body weight) did not have more chromosomal aberrations than controls. The clastogenic potential of T-2 toxin was very weak. In hamster V79 cells, DON at levels of 2–3 μg/ml or more was cytotoxic, but was non-mutagenic at the hypoxanthine-guanine phosphoribosyl transferase locus, with or without hepatocytic mediated activation (Rogers & Heroux-Metcalf, 1983).

According to Reiss (1975), DAS induced various cytological abnormalities including shortened chromosomes, enlarged nucleoli, and a few chromosome breaks in the root tips of *Allium cepa* (common onion) at concentration of 1000 and 100 μg/ml. T-2 toxin and satratoxin H were C-mitotic poisons at concentrations exceeding 10 μg/g. Typical C-mitotic action on chromosomes morphology was produced by both toxins and was comparable to that of colchicine. In addition, T-2 toxin induced polyploidy (Linnainmaa et al., 1979).

T-2 toxin and satratoxin H were not mutagenic in a sex-linked recessive lethal test in *Drosophila* (Sorsa et al., 1980). Lack of potency to produce recessive lethal mutations in *Drosophila* was consistent with negative results obtained in certain bacterial assays in which trichothecenes were inactive as both base pair and frame shift mutagens (Kuczuk et al., 1978; Ueno et al., 1978a).

II.4.2.8 Teratogenicity and reproductive effects

T-2 toxin was embryotoxic and teratogenic in mice. T-2 toxin dissolved in propylene glycol was injected intraperitoneally into pregnant mice on one of days 7–11 of gestation at doses of 0.5, 1, or 1.5 mg/kg body weight. T-2 toxin (doses of 1 or 1.5 mg/kg) caused significant maternal mortality, fetal death, and fetal body weight loss. Approximately 37% of the fetuses from dams given 1 (8 litters) or 1.5 mg (4 litters) T-2 toxin/kg on day 10 were

grossly malformed. The most frequent anomalies were bent, shortened, or missing tails, and limb malformations, including oligodactyly and syndactyly. Exencephaly, open eyes, retarded jaw, and skeletal malformations of the rib or vertebrae were also found in the fetuses (Stanford et al., 1975).

Pregnant CD-1 mice (18 litters/treatment group) were administered T-2 toxin dissolved in propylene glycol ip at 0.5 mg/kg body weight on gestation days 8 or 10. The T-2 toxin produced grossly malformed fetuses, principally with tail and limb anomalies. A higher incidence of malformations was observed when a T-2 toxin dose of 0.5 mg/kg body weight was combined with an ochratoxin A dose of 4 mg/kg body weight. An increase in fetocidal effects was found in offspring of dams in groups treated with the high-dose combination, on either day. Few skeletal and visceral malformations were noted (Hood et al., 1978). T-2 toxin (0.5 mg/kg body weight) dissolved in 1:1 mixture of propylene glycol and 0.1N sodium bicarbonate was administered ip alone or in combination with rubratoxin B (0.4 mg/kg body weight) to pregnant CD-1 mice on day 1 of gestation. Only T-2 toxin resulted in gross malformations. The combination of toxins increased the adverse effects on fetal body weight and mortality, but not the incidence or severity of the gross malformations (Hood, 1986).

The teratogenicity of orally administered T-2 toxin dissolved in propylene glycol was evaluated in a study using 350 female CD-1 mice and doses of 0.5, 1.0, 2.0, 3.0, 3.5, or 4.0 mg/kg body weight given on day 9 of gestation with a single dose of 3.0 mg/kg on day 6, 7, 8, 10, 11, or 12 of gestation. In the first study, the doses of 3.5 and 4.0 mg/kg produced maternal deaths and toxicity; no fetuses were produced by the dams in the 4.0 mg/kg dose group and significantly fewer fetuses were produced by dams in the 3.5 mg/kg dose group. More major and minor defects were seen in offspring in the 3.0 mg/kg dose group. In the second study, the treated females had greater fetal loss than controls and the greatest number of dead fetuses occurred among litters treated on day 9 of gestation. Major skeletal defects were more numerous in mice treated on day 7 of gestation. The results indicated that a single oral dose of T-2 toxin in propylene glycol was primarily maternally toxic and embryolethal; defective

development was possibly secondary to maternal toxicity (Rousseaux & Schiefer, 1987).

Fusarenon-X was embryotoxic, but not teratogenic (Ito et al., 1980). Fusarenon-X dissolved in saline was given to pregnant DDD mice by subcutaneous injection at doses of 0.63, 1.0, 1.6, 2.6, or 4.1 mg/kg body weight, or by feeding at concentrations of 5, 10, or 20 mg/kg diet during pregnancy. Two dams given a single sc dose of 4.1 mg/kg died within 24 h of injection. Abortion was induced in all females by a single injection of 2.6 mg/kg on day 10 of gestation. Smaller doses (0.63–1.6 mg/kg) produced a 16–20% abortion rate, when given on day 10. Multiple doses (8–12 or 8–14 days of gestation) of 1.0 or 1.6 mg/kg produced 100% abortion. When the mice were fed a diet containing 5, 10, or 20 mg fusarenon-X/kg throughout the gestation period or in the early stages of gestation, the mycotoxin inhibited embryonal implantation. Feeding fusarenon-X at 20 mg/kg for 7 days during the middle stages of gestation induced abortion in 100% of dams. Fetal body weight was significantly reduced by the administration of the mycotoxin, but no significant teratogenic effects were observed in the fetuses of dams in either the subcutaneous injection or feeding study (Ito et al., 1980).

The effects of NIV on fertilization, course of pregnancy, and fetuses were examined in ICR mice. In a study by Ito et al. (1986), pure NIV was injected ip in pregnant mice (groups of 10 animals each), at dose levels of 0, 0.1, 0.5, or 1.5 mg/kg body weight per day, on days 7–5 of gestation. The highest dose caused stillbirths after vaginal haemorrhage in 6 out of 10 animals. High embryo lethality was recorded in the 2 highest dose groups (88 and 48%). No fetal malformations were observed in the treated groups. A single administration of 3 mg/kg on day 7 affected the embryo within 10 h, damaged the placenta within 24 h, and caused stillbirths at 48 h.

While NIV is embryotoxic, it is not teratogenic (Ito et al., 1988). Thirty ICR mice in 3 batches of 10 animals each were fed diets mixed with mouldy rice powder containing NIV at final levels of 6, 12, or 30 mg/kg feed per day throughout gestation. There were 11 controls. Purified NIV was also administered by gavage to 35 animals in 4 groups at doses of 1–20 mg/kg body weight on days

7–15 of gestation. There were 10 controls. Embryotoxicity associated with maternal weight loss was observed in the groups receiving 30 mg/kg diet and 10 mg/ kg body weight per day, by gavage, whereas lower levels, such as 5 mg/kg body weight per day, by gavage, did not have any embryotoxic effects. Intrauterine growth retardation was found at term in the fetuses of mice exposed to 12 mg/kg feed and 5 mg/kg by gavage. NIV did not have any significant adverse effects on the incidence of gross skeletal and visceral malformations. As 5 mg/kg body weight per day given by gavage corresponds to a feed level of approximately 35 mg/kg feed, the above data indicate that exposure to 30 mg NIV/kg feed throughout the gestation period results in embryotoxicity. However, exposure to approximately 35 mg/kg feed during days 7–15 only of the gestation period does not induce embryotoxic effects, which shows the significance of constant exposure versus intermittent exposure to NIV.

DON was embryotoxic and teratogenic when dissolved in distilled water and given for 4 consecutive days (days 8–11 of gestation), by oesophageal intubation, to 15–19 pregnant Swiss-Webster mice (Khera et al., 1982). The incidence of resorptions was 100% at doses of 10 or 15 mg/kg body weight, and 80% at 5 mg/kg body weight. The dose of 5 mg/kg reduced the number of live fetuses and reduced the average fetal weight compared with the controls. Low incidences of skeletal and visceral anomalies were found in the fetuses of the 1, 2.5, and 5 mg/kg groups. The skeletal malformations occurred in a dose-related manner and included lumbar vertebrae with fused arches or partly absent centra, and absent or fused ribs.

On the other hand, DON failed to produce embryotoxic and teratogenic effects when fed *ad lib* at 0.5, 2.0, or 5.0 mg/kg to Fisher 344 rats during the entire course of pregnancy (Morrissey, 1984). No overt signs of toxicity were observed in the dams and no changes in maternal feed consumption were observed at any dose.

DON was fed to 71 adult female New Zealand White rabbits in 7 batches of 6–14 animals during the entire period of gestation at doses of 0.3, 0.6, 1.0, 1.6, 1.8, or 2.0 mg/kg body weight. There were 25 controls. The fetal effects consisted of 100% incidence

of fetal resorption in the females fed 1.8 and 2.0 mg/kg and reduced average body weight of fetuses from dams fed 1.0 and 1.6 mg/kg. These doses were not teratogenic (Khera et al., 1986).

Weanling F_0 male and female mice were fed diets containing DON at a dose of 2.0 mg/kg body weight (15 male and 15 female animals) and 0.375, 0.75, or 1.5 mg/kg body weight (7 males and 59 female animals). There were 30 controls in the first study and 26 in the second. After 30 days of dietary feeding, the mice were allowed to mate and the pregnant females were allowed to litter normally. The F_{1a} progeny were examined up to 21 days of age and discarded. The F_0 mice were rebred. The females bred to produce the F_{1b} litters were killed on day 19 of gestation and the fetuses were examined for gross, visceral and skeletal malformations. Reductions were observed in feed intake and in the body weight of F_0 male and female mice, the number of live pups and postnatal survivors, postnatal body weight of F_{1a} progeny, number of live fetuses, and fetal body weight of F_{1b}. No adverse effects on the fertility of F_0 mice and no major malformations in F_{1b} fetuses were found. Results of cross-fostering offspring between control dams and 1.5 mg/kg dams indicated that both postnatal survival and body weight were adversely affected by prenatal exposure as well as by combined pre- and post-natal exposure (Khera et al., 1984).

Male and female Sprague-Dawley rats (3 groups of 15 male and 15 female animals each) were fed diets containing DON at levels of 0.25, 0.5, or1.0 mg/kg body weight. Controls consisted of two groups of 15 males and 15 females. After 6 weeks of feeding, the rats were bred. The mated females, maintained on their respective diets, were killed on the last day of pregnancy and fetuses were evaluated for effects on pre-natal development. No adverse effects were observed except for dilatation of the renal pelvis and urinary bladder (Khera et al., 1984).

Male (20/group) and female Sprague-Dawley rats (25/group) were fed a diet containing 20 mg purified DON/kg for 60 and 15 days, respectively, before mating. Rats consuming the DON-supplemental diet throughout gestation and lactation did not show any clinical signs of toxicity, but had reduced body weights. Only 50% of the matings between toxin-fed rats resulted in

pregnancy compared with 80% in the controls. No differences were detected among the groups in sex ratio, survival rate, or average litter number and weight. Pup weight gains in all groups were comparable up to post-natal day 14. From day 14 to 21, however, male and female pups of the control group showed significantly improved weight gains compared with pups from treated dams. No treatment-related histological abnormalities were found in the testes or ovaries of treated pups (Morrissey & Vesonder, 1985).

A 2-generation reproduction and teratology study was carried out using 90 female CD-1 mice fed a semisynthetic diet containing the T-2 toxin at 1.5 or 3.0 mg/kg, concentrations considered possible under field conditions. Results indicated that continuous feeding of T-2 toxin at low concentrations had minimal, if any, toxic effects on female reproduction and fetal development and that T-2 toxin fed continuously at 1.5 or 3.0 mg/kg was not teratogenic or fetocidal and produced minimal effects on the growth rates of CD-1 mice (Rousseaux et al., 1986).

II.4.3 Biochemical effects and mode of action

II.4.3.1 Cytotoxicity

In the early stages of research on trichothecenes, DAS was isolated from *F. scirpi* as a phytotoxic principle (Brian et al., 1961). Subsequent surveys confirmed the phytotoxic nature of the trichothecenes using germination of seeds of *Brassica oleracea* L. (Ueno et al., 1971c), germination of pea seedlings (Marasas et al., 1971), growth of tobacco callus tissues (Helgeson et al., 1973), the germination of tobacco plant (*Nicotiana sylvestris*) pollen (Siriwardana & Lafont, 1978), and the auxin-promoted elongation of soybean hypocotyl (Stahl et al., 1973).

Most of the trichothecenes tested have had fungistatic activity in a wide range of species (Bamburg et al., 1968a; Bamburg & Strong, 1971; Reiss, 1973); DAS and verrucarin A were particularly potent in inhibiting growth and sporulation at concentrations of 0.5–1 mg/litre.

Many of the trichothecenes have been cytotoxic to mammalian cells, both in *in vivo* and *in vitro*. In animals administered trichothecenes, the mucosa of the stomach and small and large intestines had mucosal erosions, mucosal necrosis with ulceration, and severe haemorrhages; radiomimetic effects included necrosis of actively-dividing cells in the thymus, spleen, ovary, testis, and lymph nodes (Saito & Ohtsubo, 1974). In a cell culture system, Grove & Mortimer (1969) demonstrated the cytotoxicity of DAS and its chemically modified compounds on hepatocytes of human origin and hamster kidney cells. Ohtsubo et al. (1968), Ohtsubo & Saito (1970), and Bodon & Zoldag (1974) described the cytotoxicity of NIV and related trichothecenes for HeLa cells, and of T-2 toxin for epithelial cells of pig kidney, respectively. The macrocyclic trichothecenes, verrucarins and roridins, were highly cytotoxic for 0815 mouse tumour cells in the ng/ml range (Harri et al., 1962).

Tanaka et al.(1977), investigated the cytotoxicity of 20 types of trichothecenes (11 type A, 6 type B, 1 type C, and 2 type D) for 3 cell lines, HeLa, HEK, and HL cells. The type D trichothecenes, such as verrucarin A and roridin A had LC_{50}s in the range of 0.003–0.005 mg/litre; neosolaniol and NIV in the range of 0.1–1 mg/litre, and deoxynivalenol in the range of 1–5 mg/litre. Trichodermol, calonectrin, monoacetyldeoxynivalenol, and tetraacetylnivalenol were weakly cytotoxic within the range of 5–10 mg/litre.

Chinese hamster ovary (CHO) and African green monkey kidney (VERO) cell cultures were exposed to 0.01 or 1.0 ng T-2 toxin/ml for 1 or 12 h. The cells exhibited morphological changes considered to be related to inhibition of protein synthesis. The alterations included disassociation of polysomes and matrix density, ballooning of intracristal space, and malalignment of cristae in mitochondria. The CHO cells had bleb formations of plasma membrane, a change produced by exposure to establish inhibition of protein synthesis (Trusal, 1985).

II.4.3.2 Inhibition of protein synthesis

The initial observation that the trichothecene mycotoxins inhibited protein synthesis in mammalian cells was made by Ueno

et al. (1968). They reported that NIV produced a dose-dependent inhibition of incorporation of several amino acids into protein in rabbit reticulocytes (Ueno et al., 1968) and Ehrlich ascite tumour cells (Ueno & Fukushima, 1968). This inhibitory effect of the trichothecene mycotoxins was observed in the whole animal (Ueno, 1970), a protozoan (Ueno & Yamakawa, 1970), HeLa cells (Liao et al., 1976), cultured mammalian cells (Ohtsubo et al., 1968; Ohtsubo & Saito, 1970), rat hepatocytes (Ueno et al., 1973b), hamster ovary cells (Gupta & Siminovitch, 1978), human tonsil (Carrasco et al., 1973), yeast spheroplasts (Stafford & McLaughlin, 1973; Cundliffe et al., 1974; McLaughlin et al, 1977), rabbit reticulocytes (Ueno et al., 1968, 1969a, 1973b,c; Ueno & Shimada, 1974; Wei & McLaughlin, 1974; Mizuno, 1975; Carter et al., 1976), and lymphocytes (Hartman et al., 1978). No inhibitory effect on bacterial cells was observed (Ueno et al., 1973b).

The effects of T-2 toxin on protein and DNA synthesis were studied in Swiss mice and hepatoma cell cultures. T-2 toxin was given as a single ip dose of 0.75 mg/kg and as a 3-day and a 7-day daily treatment. The toxin inhibited protein synthesis after all 3 schedules of treatment and inhibition was present in cells obtained from bone marrow, spleen, and thymus. Protein synthesis was inhibited *in vitro* in hepatoma cell cultures and PHA-stimulated lymphocytes (Rosenstein & Lafarge-Frayssinet, 1983).

The effects of T-2 toxin on rat hepatocytes were studied in culture by the addition of several doses at either 1 or 12 h of exposure. A dose of 0.01 ng T-2 toxin/ml produced a 75% inhibition of protein synthesis within 1 h. At a higher dose of 1.0 ng/ml, hepatocytes recovered from a 1-h but not a 12-h exposure. Cell damage (release of lactate dehydrogenase) lagged behind inhibition of protein synthesis, which was 90% at the 1 ng/ml dose. Ultrastructural alterations were present in the endoplasmic reticulum and mitochondria. Degranulation involved the rough endoplasmic reticulum. Mitochondria had translucent foci and electron dense cores (Trusal & O'Brien, 1986).

NIV inhibited poly-U, poly-A, and poly-C directed incorporation of phenylalanyl-tRNA into phenylalanine without affecting the activation of amino acids. The inhibition was presumably caused by impairment of ribosomal function (Ueno et al., 1968). Fusarenon-X caused breakdown of polysomes in rabbit reticulocytes (Ueno et al., 1973b) and in mouse fibroblasts (L-cells), shortly after exposure (Ohtsubo et al., 1972). The breakdown of polysomes was consistent with the action of an inhibitor of initiation of protein synthesis. T-2 toxin, DAS, and verrucarin A, but not trichodermin, also induced the disaggregation of polysomes in HeLa cells (Liao et al., 1976). These results were consistent with the effects of an inhibitor of prolongation or termination of protein synthesis (Stafford & McLaughlin, 1973). An important point was that the trichothecenes, regardless of whether they were I-type or ET-type, interacted with the peptidyl transferase centre on the 60S ribosomal subunit and inhibited the transpeptidation of the peptide bond formation process.

Current research has focused on clarifying the molecular species of ribosomal protein that regulates the binding of trichothecene mycotoxins. A mutant of yeast (Schindler et al., 1974; Jimenez & Vazquez, 1975; Jimenez et al., 1975; Grant et al., 1976; Carter et al., 1980) and Chinese hamster ovary cells, resistant to the effects of trichothecenes on protein synthesis (Gupta & Siminovitch, 1978), have been isolated. Friend & Warner (1981) clearly demonstrated that the gene for trichodermin resistance in yeast specifies ribosomal protein L3, the largest of the yeast ribosomal proteins.

The ribosomal subunits of *Myrothecium verrucaria*, a producer of macrocyclic trichothecenes, were resistant to T-2 toxin. It can be assumed that the 60S subunits of eukaryotes are responsible for the sensitivity to the trichothecenes. (Hobden & Cundliffe, 1980).

II.4.3.3 Inhibition of nucleic acid synthesis

The dose-dependent inhibition by NIV of DNA and RNA synthesis in Ehrlich ascites tumour cells was first observed by Ueno & Fukushima (1968). Inhibition (70%) of protein synthesis was induced by 1–10 mg NIV/litre, and thymidine incorporation into

DNA was inhibited by as much as 60%; suppression (30%) of uracil incorporation into RNA was slight. Similar results were obtained with several trichothecenes, including trichodermin, diacetoxyscirpenol, and fusarenon-X, in other cultured cells, such as KB cells (Ohtsubo et al., 1968), HeLa cells (Liao et al., 1976), mouse L-cell fibroblasts (Ohtsubo et al., 1972), hamster ovary cells (Gupta & Siminovitch, 1978), and lymphocytes (Hartman et al., 1978).

The inhibition of DNA and RNA synthesis by trichothecenes required higher concentrations of toxins than the inhibition of protein synthesis and the extent of the inhibition was much less. Thus, the observed inhibition of DNA and RNA synthesis in toxin-treated animal cells was presumed a secondary effect of the trichothecenes. This hypothesis was supported by the findings of Tashiro et al. (1979) who reported that, in *in vitro* studies, a concentration of 0.0236–1.889 mmol fusarenon-X/litre did not inhibit DNA-dependent RNA hybridases of rat liver and *Tetrahymena pyriformis*.

Munsch & Mueller (1980) reported that the incorporation of ^{3}H-thymidine into DNA in cell lines from thymus was strongly inhibited by over 10 ng T-2 toxin/ml and slightly inhibited by 0.1–10 ng T-2 toxin/ml. A low concentration of 0.1–1 ng/ml toxin caused a transient increase in DNA polymerases, α- and β-terminal deoxynucleotidyl transferases. At a high dose of more than 1 ng T-2 toxin/ml, these enzymatic activities were strongly inhibited. Rosenstein & Lafarge-Frayssinet (1983) described the depression of DNA synthesis *in vivo* and *in vitro*. Three treatment schedules: a single dose, 3 daily doses, or 7 daily doses of 0.75 mg T-2 toxin/kg inhibited DNA synthesis in cell cultures from the spleen, thymus, and bone marrow of treated mice. The mycotoxin also inhibited DNA synthesis *in vitro* in cultures of hepatoma cells and in PHA-stimulated lymphocytes (Rosenstein & Lafarge-Frayssinet, 1983).

Agrelo & Schoental (1980) found that hydroxyurea did not alter unscheduled DNA synthesis in cells treated with T-2 toxin and HT-2 toxin (6 ng/ml). However, the combination of rat liver microsomes and hydroxyurea resulted in an increase in unscheduled DNA synthesis in cells exposed to 100 μg HT-2 toxin/

litre. The data of Agrelo & Schoental (1980) suggest that the microsomal drug-metabolizing enzyme system may participate in the induction of DNA damage by trichothecenes.

The effects of T-2 toxin and DAS on DNA synthesis in phytohaemagglutinin, stimulated in the peripheral blood lymphocytes of human beings, was assayed by incorporation of ^{3}H-thymidine. Total inhibition was obtained by 8 ng T-2 toxin and DAS and 80% was obtained with 1.5 ng T-2 toxin and 2.7 ng of DAS (Cooray, 1984).

Fusarenon-X and related toxins inhibited protein and nucleic acid synthesis in *Tetrahymena pyriformis* in a manner similar to that observed in cultured mammalian cells (Ueno & Yamakawa, 1970). In synchronously dividing *Tetrahymena* cells (Iwahashi et al., 1982), the incorporation of radioactive amino acids, thymidine, and uracil into protein, DNA, and RNA, respectively, was achieved by nearly the same concentration of T-2 toxin. Inhibition of protein synthesis was explained by the high affinity of T-2 toxin for 60S ribosomal subunits of *Tetrahymena*, as is the case with cultured cells. However, neither DNA and RNA synthesis nor RNA hybridase activity were altered by T-2 toxin in an *in vitro* system using isolated nuclei from normal cells or from cells pretreated with T-2 toxin. The mechanism of inhibition of nucleic acid synthesis in the *in vivo* system is not yet understood.

II.4.3.4 Alterations of cellular membranes

The trichothecenes as a group inhibit protein synthesis, but the potency of the effect varies according to the trichothecene and the system *(in vitro* or *in vivo)* used to measure the inhibition. T-2 toxin was a potent inhibitor of protein synthesis both *in vitro* and *in vivo* (Ueno et al., 1973b; Rosenstein & Lafarge-Frayssinet, 1983).

In vitro experiments were conducted to determine the effects of T-2 toxin on the entry of sucrose into bovine erythrocytes, entrapment of sucrose and inulin in carrier erythrocytes, entrapment of T-2 and binding of T-2 toxin, and the measurement of cell permeability to the entrapped T-2 toxin. At the highest concentration of T-2 toxin (20 μg), no entry of ^{14}C-sucrose or ^{3}H-inulin

was observed. Very little ^{3}H-T-2 toxin was bound to bovine erythrocytes and binding was independent of T-2 toxin concentration. The mycotoxin had no effect on the entrapment of sucrose or inulin. Carrier erythrocytes retained 85% of ^{14}C-sucrose and only 18% of ^{3}H-T-2 toxin. Thus, T-2 toxin diffused from carrier cells much more rapidly than sucrose. It was concluded that the interaction of T-2 toxin with bovine erythrocytes was minimal and intercalation with the inner bilayer was not likely, because the increase in cell volume that would have resulted did not occur (DeLoach et al., 1987).

The effects of T-2 toxin on membrane function were studied using L-6 myoblasts. The minimal effective concentration (MEC) of T-2 toxin for reduction in uptake of calcium and glucose and for reduction in uptake of leucine and tyrosine and their incorporation into protein was 4 pg/ml. The uptake of rubidium was increased at 0.4 pg/ml and reduced at 4 pg/ml or more. Thymidine uptake and incorporation into DNA had a biphasic response with an increase at 0.4 pg/ml and a reduction at 4 pg/ml for uptake and 40 pg/ml for incorporation. Calcium efflux was reduced after 1, 5, and 15 min exposure to T-2 toxin at a concentration of 40 pg/ml. These data indicate that T-2 toxin has multiple effects on all membrane function at very low concentrations and that these effects are independent of inhibition of protein synthesis (Bunner & Morris, 1988).

II.4.3.5 Other biochemical effects

Fusarenon-X inhibited the uptake of phosphate by *Tetrahymena* cells (Chiba et al., 1972). It caused a 50% inhibition of incorporation of acetate into phospholipids and a ten-fold stimulation of incorporation into triglyceride, effects that may be secondary to inhibition of phosphate uptake. However, in rabbit reticulocytes, fusarenon-X, T-2 toxin, and neosolaniol at concentrations of 100 mg/litre did not inhibit Na^{+}-dependent glycine transport (Ueno et al., 1978b).

When SH-enzymes were pre-incubated with selected trichothecenes in the absence of substrates, the activities of such enzymes as creatine phosphokinase and lactate and alcohol dehydrogenases were reduced (Ueno & Matsumoto, 1975).

However, neither urease activity (Reiss, 1977) nor rat liver lysosomal cathepsin activity (Farb et al., 1976) was affected by diacetoxyscirpenol or T-2 toxin, respectively, *in vitro*. Foster et al. (1975), reported the reactivity of T-2 toxin with glutathione in the presence of epoxide-S-GSH-transferase.

When mice were exposed to trichothecene mycotoxins, no detectable alterations were observed in hepatic and renal functions. In chicks fed a diet containing 10 mg T-2 toxin/kg for 3 weeks, a hepatic microsomal aminopyrine, demethylase, was reduced by 29% compared with the control, while another mixed function oxidase, aniline hydroxylase, was not significantly affected (Coffin & Combs, 1981). In mice receiving a sublethal dose of fusarenon-X ip, a rapid hypoglycemia was followed by depletion of hepatic glycogen (Shimizu et al., 1979). The authors suggested that the toxin had induced a malfunctioning of glucose absorption in the intestines and had accelerated glycolysis. However, the trichothecene mycotoxins are highly cytotoxic to the epithelia of the intestine, and impairment of carbohydrate metabolism and absorption may be a secondary effect of this cytotoxicity.

II.4.4 Structure-activity relationships

After the isolation and identification of numerous derivatives of the trichothecenes, the structure-activity relationship was investigated, on the basis of the information on lethal toxicity, dermal toxicity, cytotoxicity, inhibition of protein synthesis, and association with ribosomes.

The 12,13-epoxide of the trichothecenes is essential for their biological activity. The de-epoxidation of DON and T-2 toxin by rumen-microorganisms (King et al., 1984) and in mammalian systems (Yoshizawa et al., 1983) results in loss of toxicity.

The macrocyclic ring contributes to the highly lipophylic property of the trichothecenes, and the macrocyclic trichothecenes, such as verrucarins, roridins, satratoxins, and baccharins, exhibit a potent cytotoxicity towards cultured mammalian cells (Kupchan et al., 1976, 1977; Jarvis et al., 1978, 1980).

In contrast, the polyhydroxylated alcohols, such as verrucarol, NIV, and DON, are highly hydrophilic, resulting in a decrease

in cytotoxicity and dermal toxicity compared with their parent trichothecenes (Ueno et al., 1970; Wei et al., 1974).

II.4.5 Prevention and therapy of trichothecene toxicosis

In studies to investigate effects of various dietary supplements on T-2 toxin toxicity, weanling male Wistar rats in groups of 10, with adequate controls, were fed diets containing 5% each of bentonite, anion exchange resin, cation exchange resin, or vermiculite-hydrobiotite with and without 3 μg T-2 toxin/g feed, for 2 weeks. Bentonite and anion exchange resin were most effective in reducing the growth depression and feed refusal caused by T-2 toxin. In a second study in which bentonite and anion exchange resin were administered at levels of 2.5, 5.0, 7.5, or 10% of diet, 10% bentonite in the diet was the most effective dietary supplement. Dietary bentonite reduced the absorption of ^{3}H-T-2 toxin and increased faecal elimination (Carson & Smith, 1983).

Smectite, a clay composed of insoluble silicates of aluminum and magnesium, incubated with T-2 toxin for 24 h before the toxin was administered orally to mice(1 mg/kg per day), prevented the acceleration of gastric emptying and transit time for a milk meal, usually induced by administration of T-2 toxin. Smectite given together with the toxin did not prevent the gastrointestinal effects of T-2 toxin (Fioramonti et al., 1987). Male Sprague-Dawley rats were given 15 or 5 mg/kg body weight of a platelet activating factor antagonist, with and without T-2 toxin, by intravenous injection. The drug prolonged the survival of conscious rats exposed to 0.65 mg T-2 toxin/kg (Feuerstein et al., 1987).

The effects of certain drugs and metabolic inhibitors on the toxicity of T-2 toxin have been studied in male ddY mice. The toxin at a dose of 1.8 mg/kg was given subcutaneously and the drugs and other chemicals were given ip. The lethal effects were reduced by the steroid drugs, prednisolone and dexamethasone; survival times were increased by the antihistaminic drug diphenhydramine, and an opioid antagonist, naloxone. Prednisolone also reduced the leukocytosis that developed after T-2 toxin treatment and decreased the increase in ear weight caused by

the mycotoxin. Other antihistaminic and/or antiserotonic drugs used in the treatment were found not to be effective in preventing the lethal effects of T-2 toxin (Ryu et al., 1987).

Male ddY mice and male Wistar rats in several groups of 10 animals each, pretreated with a radioprotective compound or with an anti-inflammatory agent, were given T-2 toxin or fusarenon-X (1.5 mg/kg body weight). Out of the 20 compounds evaluated, only prednisolone and hydrocortisone (100 mg/kg ip) were effective. They reduced mortality from 90% in controls to less than 30% and they also reduced the trichothecene-induced increase in intestinal fluid volume (Mutoh et al., 1988).

Cutaneous irritation produced by T-2 toxin in 10 Porton female rats was largely reduced by application, within 10 min, of an aqueous soap solution when the dose was low (1.0 $\mu g/cm^2$), but the solution was largely ineffective over a time span of 60 min when the dose was higher (100 μg T-2 toxin). Washing the site of application with polyethylene glycol 300 was very effective in removing even large doses (100 μg) of T-2 toxin from the skin (Fairhurst et al., 1987).

The median effective dose of oral superactive charcoal in preventing deaths in batches of 5 female Sprague-Dawley rats was 0.175 g/kg. A dose of 1 g superactive charcoal/kg body weight increased survival times and rates in rats given the lethal dose of 8 mg T-2 toxin/kg as long as 3 h after the toxin was administered by gavage (Galey et al., 1987).

II.5 EFFECTS ON MAN

II.5.1 Contemporary episodes of human disease

Two outbreaks of trichothecene-related disease have been reported, one in China in 1984/85 (Luo, 1988) and one in India in 1987 (Bhat et al., 1989). Each involved several hundred cases.

During the first incident, outbreaks of mouldy corn and scabby wheat poisoning were reported. Out of approximately 600 persons who consumed mouldy cereals, there were 463 cases of poisoning (77% of the total). The latency period for the onset of symptoms was 5–30 min. These included nausea, vomiting, abdominal pain, diarrhoea, dizziness, and headache. No deaths occurred. Pigs and chicks fed the same mouldy cereals were also affected (Luo, 1988). GC-MS and RIA were used in the analysis of 5 samples of the mouldy corn. DON was detected within a range of 0.34–92.8 mg/kg and zearalenone within a range of 0.004–0.587 mg/kg. T-2 toxin and NIV were not found. TLC was used in the analysis of 19 samples of scabby wheat collected from the affected and non-affected families. The DON content was 1.0–40.0 mg/kg, which was significantly higher than that in the non scabby wheat samples. In addition to DON, zearalenone was detected in 2 samples, the contents of which were 0.25 and 0.5 mg/kg, respectively. No T-2 toxin was found in the samples (Luo 1988).

An analogous outbreak was reported in Kashmir, India, in 1987 (Bhat et al., 1987, 1989). It was ascribed to the consumption of bread made from flour that had become mouldy in storage following unseasonal rains in the wheat-harvesting season, from which *Fusarium* sp. was grown, and which were found to contain mycotoxins. Of the 224 persons investigated on a random sample basis, 97 were affected with symptoms including abdominal pain (100%), throat irritation (63%), diarrhoea (39%), blood in stools (5%), and vomiting (7%). Symptoms developed 15 min to one hour after consumption of locally baked bread. In 12 out of 24 samples of refined wheat flour used in the preparation of bread,

the following mycotoxins were found: DON (0.35–8.38 mg/kg), Ac-DON (0.64–2.49 mg/kg) (no details of estimation of this derivative were available), NIV (0.03–0.1 mg/kg) and T-2 toxin (0.55–0.8 mg/kg). Quantitative estimation of DON, Ac-DON, and NIV was obtained using HPLC, and that of T-2 toxin by TLC (Bhat et al., 1987) but no rigorous confirmation of identity was undertaken. Since no fatalities occurred in either of the above outbreaks, no information is available on the patho-logical changes, if any, at autopsy.

II.5.2 Historical *Fusarium*-related diseases

In the period 1931–47, a human disease known as alimentary toxic aleukia (ATA) occurred in the USSR that was suggested to be related to the presence of toxic *Fusarium* species in mouldy overwintered grain. Data have been reviewed by Sarkisov et al. (1944) and more recently by Bilai (1977), Leonov (1977), and Joffe (1986). An association was established with the ingestion of grain invaded by some moulds, in particular *Fusarium poae* and *F. sporotrichioides*. The dominant pathological changes were necrotic lesions of the oral cavity, the oesophagus, and stomach and, in particular, a pronounced leukopenia. The primary lesion was bone marrow hypoplasia and aplasia. The disease was lethal in a high proportion of cases.

The clinical symptoms reported in ATA, as well as the identified occurrence of *Fusarium* in foodstuffs, suggest that it might have been associated with mycotoxins, identified years later in fungal cultures of *Fusarium* species under laboratory conditions, such as T-2 toxin (Mirocha & Pathre 1973) or wortmannin (Mirocha & Abbas 1989).

Scabby grain toxicosis is a disease of human beings as well as farm animals, and was reported from Japan and Korea in the period 1946–63 (Hirayama & Yamamoto 1948, 1950; Nakamura et al., 1951; Tsunoda et al., 1957; Cho 1964; Ogasawara 1965; Chung 1975). The common clinical symptoms were nausea, vomiting, diarrhoea, and abdominal pain. All cases were acute with recovery within a few days and no lethal cases were encountered. *Fusarium* fungi, *F. graminearum* in particular, were

isolated from suspected cereals (Tochinai, 1933; Tsunoda et al., 1957).

When compared with the symptoms observed in experimental animals, features of both the above human diseases were similar to trichothecene toxicosis, notably symptoms caused by DON and NIV, DAS, and T-2 toxin. However, no epidemiological studies have been reported that link ATA and scabby grain toxicosis to these chemicals.

II.5.3 Skin irritation

The Task Group was aware of several reports describing various effects on the skin of crude extracts of fungal cultures or solutions containing T-2 toxin, as well as other possible substances (Bamburg & Strong, 1971; Saito & Otshubo, 1974). These cases, all of them accidental, involved a very limited number of persons who developed severe irritation, loss of sensitivity, and desquamation. Despite the presence of T-2 toxin in the contact material, there is no evidence that the involvement of other compounds can be ruled out.

II.5.4 Studies of haemostasis

Platelet function and electron microscopic morphological changes following T-2 toxin administration were studied on platelets isolated from 12 healthy human volunteers. When platelets were incubated with T-2 toxin at doses of 5–500 $\mu g/10^9$ platelets for 20 min, there was a dose-related inhibition of platelet aggregation with different activators, including epinephrine, arachidonic acid, and collagen, and a release of dense bodies consisting mainly of serotonin-containing granules. There was also a change in membrane permeability, but no changes in shape. No correlated inhibition of thromboxane synthesis, or significant alterations in platelet calcium content were observed. The microtubular system was unaffected. It was suggested that the above observations , notably suppressed aggregation, played a contradictory role in the haemorrhagic phenomena associated with these toxins in man and animals (Yarom et al., 1984a).

II.5.5 Airborne trichothecene-related diseases

High concentrations of spores of *Stachybotrys atra* were discovered in the air of living rooms of a suburban house in Chicago, USA (Croft et al., 1986). Over a period of several years, the 5 occupants of the house had suffered a variety of non-specific symptoms including signs of colds, sore throats, diarrhoea, headaches, dermatitis, intermittent focal alopaecia, and generalized malaise. Chemical analysis of building materials supporting the growth of *Stachybotrys atra* confirmed the presence of the macrocyclic trichothecenes, verrucarin B and J, satratoxin H, and trichoverrins A and B. Five weanling Sprague-Dawley rats and 5 mice, administered extracts of contaminated materials orally, died within 24 h of exposure, whereas control animals remained unaffected. Histological lesions of the animals tissues were degeneration, necrosis and haemorrhage of the brain, thymus, spleen, intestine, lungs, heart, lymph nodes, liver, and kidneys. Although the clinical symptoms could have been related to allergic responses, the isolation of potent mycotoxins suggests that they were the causal factor of the illness observed.

II.5.6 Toxicological information on man, obtained from therapeutic uses

DAS (Anguidine) has been undergoing clinical trials as a chemotherapeutic agent in cancer patients. During a weekly schedule of DAS, using healthy volunteers as controls, a dose of 5 mg/m^2 body surface infused over 3 h, produced nausea, vomiting, hypotension, neurological symptoms (confusion, hallucinations and psychomotor seizures), chills, fever, and diarrhoea (DeSimone et al., 1979). A similar study carried out on 20 other cancer patients revealed gastrointestinal and neurological toxic effects (Belt et al., 1979). A bolus administration or rapid infusion of the drug also caused gastrointestinal and neurological symptoms (Thigpen et al., 1981). The human haematopoietic system appears to be extremely sensitive to DAS. Myelosuppression was the dose limiting adverse effect of prolonged infusions over 8 h (Thigpen et al., 1981). A mean myelosuppressive dose level in the above investigation was, 5 mg/m^2 body surface by 8-h infusion or,

roughly calculated in terms of body weight, 0.2 mg/kg (0.025 mg/kg per h).

II.6 EVALUATION OF THE HUMAN HEALTH RISKS

On the basis of the data made available to the Task Group, there is a possible association between trichothecene exposure and episodes of human disease. According to the limited data available, the most frequently reported trichothecenes involved in episodes of human exposure are DON and NIV. In the episodes of alimentary toxic aleukia and scabby grain toxicosis reported in the past, a possible etiological role of trichothecenes cannot be excluded. Exposure occurs through the ingestion of contaminated food, mainly cereals. Processing, milling, and baking are not effective in destroying DON, NIV, and T-2 toxin. There is very limited evidence of exposure through inhalation, but such a possibility cannot be ruled out.

Among the naturally occurring trichothecenes in foods, T-2 toxin is the most potent, followed by DAS and NIV; DON was the least toxic in acute toxicity studies. In experimental animals, T-2 toxin and DAS produce acute-systemic effects, with necrosis of epithelial tissues and suppression of haematopoiesis. In contemporary outbreaks of disease, only gastrointestinal symptoms have been reported.

DON was shown to be teratogenic in mice but not in rats. According to published chronic toxicity studies, NIV and T-2 toxin are not tumorogenic in animals. No long-term carcinogenicity studies on DON have been published. Certain trichothecenes, such as T-2 toxin and DON, have an immunosuppressive action in animals and have produced alterations in both cell-mediated and humoral immunity. There is no evidence of immunosuppressive action in man.

Reported cases of human disease associated with trichothecene exposure are limited in number and information. Symptoms of digestive disorders and throat irritation develop rapidly after ingestion of food contaminated with trichothecenes. At present, there is no evidence of human cancer caused by trichothecenes. No reports were available to the Task Group on secondary

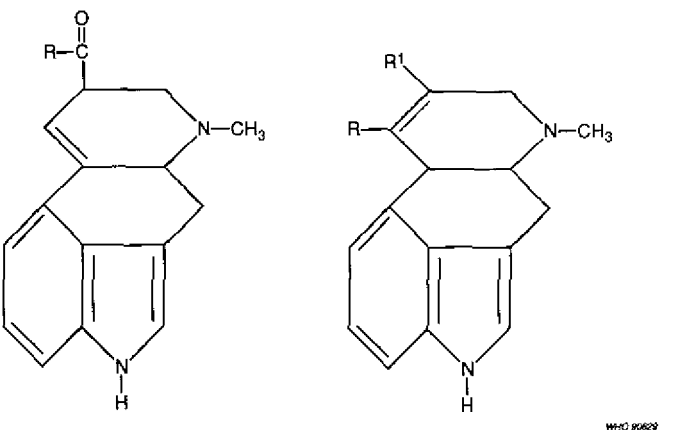
infection, by bacteria, fungi, or viruses, in human beings following trichothecene exposure, as has been observed in experimental animal studies. It appears that adequate studies elucidating such a sequence have not been made.

III. ERGOT

III.1 PROPERTIES AND ANALYTICAL METHODS

III.1.1 Chemical properties

Ergot is the French word for a rooster's spur and is used as the common term for sclerotia of fungal species within the genus *Claviceps*, in particular *C. purpurea*. The fungi infect the florets of grasses and cereal, replacing the florets with compact fungal structures, sclerotia, 2–20 mm long, somewhat curved, tapering off at the ends, and strongly coloured, often purple-black, thus resembling rooster's spurs. The sclerotia contain a large number of biologically active alkaloids, as well as amino acids, carbohydrates, lipids, and pigments, and, when the sclerotia are consumed by man and animals, toxicosis develops, called ergotism. The topic has been reviewed by Bove (1970), Van Rensburg & Altenkirk (1974), Lorenz (1979), and Berde & Schild (1978). This section deals with ergot as a toxic food contaminant; ergot compounds used as pharmaceutical drugs are excluded.



Derivatives of lysergic and isolysergic acid

Derivatives of dimethylergolines (clavines)

Fig. 4. General structure of ergot alkaloids (ergolines).

Ergot alkaloids (ergolines), of which more than 40 have been isolated from *Claviceps* sclerotia, are derivatives of lysergic acid (Fig. 4), and can be divided into 3 groups:

Group I:	Derivatives of lysergic acid, e.g., ergotamine.
Group II:	Derivatives of isolysergic acid, e.g., ergotaminine.
Group III:	Derivatives of dimethylergoline (clavines), e.g., agroclavine.

Physical and chemical properties of some ergolines are listed in Table 23.

III.1.2 Analytical methods for ergot and ergot alkaloids

III.1.2.1 Ergot

Sclerotia of *Claviceps* species are all rich in triglycerides, but only a few species have a sufficiently distinctive fatty acid composition in the sclerotia to provide a basis for identification. Sclerotia of *C. purpurea* are unique among microorganisms in containing a large (approximately 30%) triglyceride fraction in which ricinoleic acid is the principal (30–40%) component. Thus, the presence of ricinoleate in a foodstuff known to be free from other sources of ricinoleic acid (e.g., castor oil) is diagnostic for the presence of *C. purpurea* sclerotia. The sample is saponified, the free fatty acids thereby released are methylated with diazomethane, and the resulting methyl esters are analysed using gas-liquid chromatography (Mantle, 1977a). As little as 0.3% ergot in 1–2 g foodstuff has been detected by this procedure.

III.1.2.2 Ergot alkaloids

Sclerotia may contain up to 1% of total ergot alkaloids. Lysergic acid derivatives can easily be epimerized at C-8 to give isolysergic acid derivatives (Group III), thereby removing most of their biological activity. As this may happen to a variable

Table 23. Physical and chemical properties of selected ergot alkaloids (ergolines)[a]

Ergoline	Molecular formula	Melting point (°C)	$[\alpha]_D^{20}$
Group I. Derivatives of lysergic acid			
ergotamine	$C_{33}H_{35}O_5N_5$	180	-160
α-ergocryptine	$C_{32}H_{41}O_5N_5$	212-214	-190
ergocristine	$C_{35}H_{39}O_5N_5$	160-175	-183
ergosine	$C_{30}H_{37}O_5N_5$	220-230	-183
ergocornine	$C_{31}H_{39}O_5N_5$	182-184	-188
ergometrine	$C_{19}H_{23}O_2N_3$	162	+41
Group II. Derivatives of isolysergic acid			
ergocristinine	$C_{35}H_{39}O_5N_5$	226	+366
ergometrinine	$C_{19}H_{23}O_2N_3$	196	+414
ergosinine	$C_{30}H_{37}O_5N_5$	228	+420
ergocorninine	$C_{31}H_{39}O_5N_5$	228	+409
α-ergocryptinine	$C_{32}H_{41}O_5N_5$	240-243	+480
ergotaminine	$C_{33}H_{35}O_5N_5$	241-243	+369
Group III. Derivatives of dimethyloergolines (clavines)			
agroclavine	$C_{16}H_{18}N_2$	206	-151
elymoclavine	$C_{16}H_{18}ON_2$	249	-109
chanoclavine	$C_{16}H_{20}ON_2$	222	-240
penniclavine	$C_{16}H_{18}O_2N_2$	222	+153
setoclavine	$C_{16}H_{18}ON_2$	229-234	+174

[a] From: Van Rensburg & Altenkirk (1974) and Lorenz (1979).

extent during extraction, it is difficult to know whether the small proportion of isolysergic acid derivatives, commonly found during analysis of extracts from sclerotia, has been generated in whole or in part during extraction (Mantle, 1977b).

A method for the determination of *C. purpurea* ergot alkaloids in flour has been developed, based on liquid chromatography following extraction of the flour by a mixture of methylene chloride, ethyl acetate, methanol, and 28% ammonium hydroxide solution (Scott & Lawrence, 1980). After filtration and evaporation to

dryness, the residue is dissolved in methanol-ether and extracted with 0.5 N hydrochloric acid. The acid layer is washed with hexane, made alkaline, re-extracted with methylene chloride, evaporated, and the residue dissolved in methanol, ready for liquid chromatographic analysis, with identification based on fluorescence at an excitation wavelength of 235 nm. The method has a recovery of 66–93%, based on analysis of flour spiked with Group I alkaloids (ergotamine, ergocryptine, ergocristine, ergosine, ergocornine, ergometrine).

A method using high-performance liquid chromatography for the analysis of human blood has been reported (Zorz et al., 1985). Samples (5 ml) of human plasma are extracted with benzene-toluene-ethyl-acetate-diethylamine, the solvent layer separated and evaporated to dryness. High-performance liquid chromatography is performed with excitation wavelength at 285 nm for naturally occurring ergot alkaloids. According to recovery studies, concentrations as low as 0.2 mg/litre plasma can be detected.

A radioimmunoassay has been developed for determination of ergotamine and ergocristine (Arens & Zenk, 1980). The alkaloids were conjugated with bovine serum-albumin, and antibodies raised in rabbits. Using ^{3}H-labelled tracers, levels as low as 3.5 pmol of ergotamine and 0.8 pmol of ergocristine could be measured. The antibodies were highly specific, and simple lysergic acid derivatives and clavines did not cross-react. The procedure has been used in the detection of sclerotia of *C. purpurea* with a high alkaloid concentration for industrial bioproduction.

A liquid chromatographic method for the detection of ergotamine, ergotaminine, and ergocristine in human plasma has been developed using extraction at pH 9, clean-up, and an ODS-hypersil reverse-phase column (Edlund, 1981). Levels of ergolines as low as 0.1 mg/litre in 3 ml plasma samples can be detected, with a recovery of 79–99% for the 3 ergolines.

Ergot contamination of pearl millet, due to infection by *C. fusiformis*, is characterized by the presence of clavine alkaloids (Group III). A procedure for its determination has been developed using thin-layer chromatographic separation and spectrophotometric detection following colour reaction using

Van Urk's reagent (Bhat et al., 1976; Krishnamachari & Bhat, 1976). The procedure includes defatting of the grain sample, mixing of the defatted material with ammonium hydroxide, extraction with diethyl-ether followed by extraction of the diethyl-ether phase with 0.1 N sulfuric acid. The extract is made alkaline and extracted with chloroform, followed by thin-layer chromatographic separation.

III.2 SOURCES AND OCCURRENCE

III.2.1 Fungal producers

Sclerotia are compact hyphal structures that develop in the colonies of many fungal genera. The sclerotia of species within the genus *Claviceps* are unique in terms of size (length up to several cm), pronounced colour, and because the sclerotia of several species contain highly biologically active compounds, the alkaloids. The sclerotia develop during the infection of plants; host plants for the *Claviceps* species mainly belong to the grass family (Gramineae), which comprises the true cereals. However, a few plant species within the family Juncaceae and Cyperaceae can also act as hosts. Most *Claviceps* species have a monogeneric host range, but *C. purpurea* is unique in that it has a very wide host range (Van Rensburg & Altenkirk, 1974; Lorenz, 1979). The eight leading cereals produced in the world are wheat, rice, corn, sorghum, rye, barley, oats, and millet, and they can all be hosts for *Claviceps* species. Man and animals are exposed to toxic sclerotia from two species, *C. purpurea* and *C. fusiformis*; farm animals are also exposed to toxic sclerotia from *C. paspali*, growing on grass. Sclerotia of *C. purpurea* have the dimension of 2–20 × 1–6 mm and are purple-black in colour, whereas *C. fusiformis* has small (2 × 4 mm) purple-red coloured sclerotia (Loveless, 1967; Siddiqui & Khan, 1973; Mantle, 1977b). *C. purpurea* primarily attacks cross-pollinated species in which the florets tend to remain open for a relatively long time and in which sterility occurs.

The cereals most commonly contaminated with ergot from *C. purpurea* are rye, wheat, triticale (the cross-breed between wheat and rye), barley, oats, and sorghum. The ergot of *C. purpurea* contain Group I and II ergolines as the principal ergot alkaloids. *C. fusiformis* is a parasite of pearl millet (*Pennisetum typhoideum*) in Africa and East Asia; in India, the cereal is called bajra. Ergot of *C. fusiformis* contain predominantly Group III ergolines.

Ergot alkaloids have been isolated from fungi outside the genus *Claviceps* (*Aspergillus fumigatus, A. clavatus, A. nidulans, Rhizopus nigricans, Penicillium chermesinum, P. concavo-rugulosum, P. sizovae*) and from higher plants (*Rivea corymbosa, Ipomoea violacea, I. argyrophylla, I. hildebrandtii, I. tricolor*) (Van Rensburg & Altenkirk, 1974; Kozlovsky & Reshetilova, 1984). Whether these sources represent human exposure is not known at present.

Successful attempts have been made to produce ergot alkaloids using *C. purpurea* cultures in liquid media under laboratory conditions, and new alkaloids have been identified (Bianchi et al., 1982).

III.2.2 Biosynthesis

The ergoline ring system in ergot fungi is built up from L-tryptophan and mevalonic acid (Fig. 5). The *N*-methyl group of the

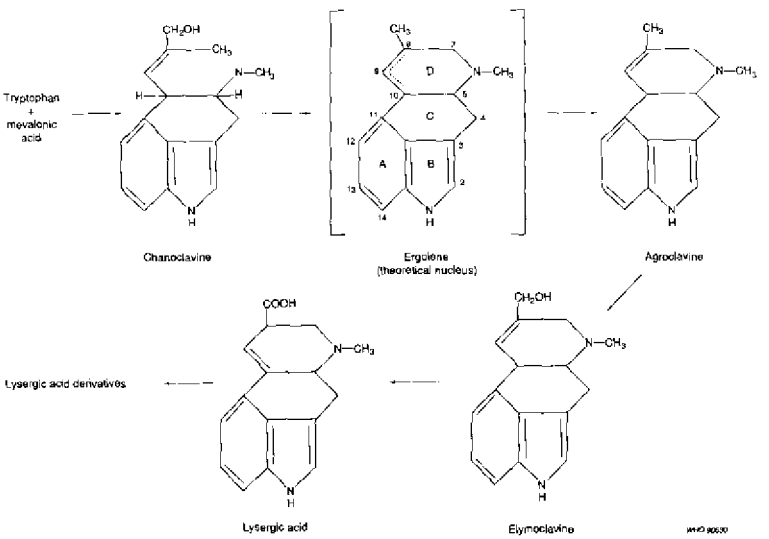


Fig. 5. Biosynthesis of ergot alkaloids (ergolines).

ergot alkaloids is derived from methionine via a transmethylation reaction. The precursors of tryptophan are indole, anthranilic acid, and indolpyruvic acid (Van Rensburg & Altenkirk, 1974; Mantle, 1977b). It appears that Group III ergolines are intermediates in the production of Group I and II ergolines by *C. purpurea*.

III.2.3 Occurrence in foodstuffs

Traditionally, contamination of grain with ergot has been expressed as a percentage on a weight basis, without measurement of total and individual amounts of ergolines. Thus, it is generally recommended that feed containing more than 0.1% ergot should not be given to animals (Young, 1981a,b). Quantitative analysis for total and individual ergolines in 14 samples of rye and wheat flour (Scott & Lawrence, 1980) indicated contamination with 6 Group I ergolines (ergometrine, ergosine, ergotamine, ergocornine, ergocryptine, ergocristine) at concentrations ranging from 0.3 to 62 μg/kg for individual ergolines. Ergocristine was the major ergoline present in the flours, 62 μg/kg being the maximal concentration found. In 17 samples of rye grain collected from health shops, 6 contained ergot; ergoline concentration and composition were not indicated (Akerstrand, 1980). In a survey of ergolines in ergot-contaminated cereals, in North America, the average total ergolines content was 0.24% (Young, 1981a,b; Young & Chen, 1982). On average, the pooled ergoline composition in sclerotia in rye, wheat, and triticale was as follows: ergocristine (31%), ergocristinine (13%), ergotamine (17%), ergotaminine (8%), ergocryptine (5%), ergocryptinine (3%), ergometrine (5%), ergometrinine (2%), ergosine (4%), ergosinine (2%), ergocornine (4%), and ergocorninine (2%). The individual ergoline composition was uniform throughout a single sclerotium or in different sclerotia from the same head, somewhat less uniform between different fields throughout a region, and highly variable from head-to-head in a given field. In contrast, the total ergoline content was highly variable within-sclerotium, within-head, head-to-head, and on a field-to-field basis. In a comparative study of ergoline in ergots from rye and wheat (caused by infection with *C. purpurea*) and in ergots from pearl millet (caused by infection with *C. fusiformis*) in South-East

Asia, it was found that the total ergoline content in the sclerotia of pearl millet was much lower (320 mg/kg) than that in the sclerotia of rye (700 mg/kg) and wheat (920 mg/kg) (Bhat et al., 1976). The ergolines in the sclerotia of pearl millet were reported to comprise agroclavine, elymoclavine, chanoclavine, penniclavine, and setoclavine.

In a survey of cereals and cereal products on the Swiss market, using a high-performance liquid chromatography procedure, the following average concentrations of total ergolines were found: wheat flour, 4.2 μg/kg; wheat flour (coarse), 30.7 μg/kg; wheat flour (more coarse), 103.4 μg/kg; rye flour, 139.7 μg/kg; and "bioproducts", 10.2–22.7 μg/kg (Baumann et al., 1985). The daily intake of total ergolines by human beings in Switzerland was estimated to be 5.1 μg/person.

III.2.4 Fate of ergolines during food processing

Treatment of sclerotia from wheat with chlorine (1%) and heat (150–200 °C) resulted in a 90% reduction in ergoline content within 4 h (Young et al., 1983). The reduction affected all the ergolines (ergotamine, ergocornine, ergocryptine, ergosine, ergometrine) in identical ways. Autoclaving sclerotia at 121 °C for 30 min resulted in a 24.6% reduction in total ergoline content. In the baking of bread and pancakes using grain that contained naturally occurring ergot, a 59–100% reduction in the individual ergolines (ergosine, ergocornine, ergometrine, ergotamine, α-ergocryptine, ergocristine) was observed in whole wheat bread, a 50–86% reduction in all-rye flour bread, and a 25–74% reduction in triticale pancakes (Scott & Lawrence, 1982).

When bread made of rye flour spiked with finely ground sclerotia of *C. purpurea*, (continuing a total ergoline concentration of 312.8 μg/kg), was baked, there was an overall reduction of 50% in the ergoline concentration, as measured by high performance liquid chromatography (Baumann et al., 1985).

III.3 METABOLISM

No published information is available on the metabolism in animals or human beings of ergots containing ergolines and combinations of individual ergolines.

III.4 EFFECTS ON ANIMALS

III.4.1 Field studies

An outbreak of bovine abortion associated with the ingestion of ergot was reported by Appleyard (1986).

Eleven out of 36 suckler cows, all in late pregnancy, aborted in 7–11 days following introduction to a rye grass pasture heavily infested with ergot. At least 25% of the rye seed heads contained sclerotia of *C. purpurea*, with up to 8 sclerotia present on any one seed head. The sclerotia contained 1.57 mg total ergolines/g, consisting of 67% ergotamine and 17% ergotaminine, and smaller amounts of ergometrine. Ergocryptine and ergosine were also present together with their corresponding -inine isomers.

Ten out of the 11 calves were delivered dead. None of the aborting cows showed any premonitory signs of calving and, after parturition, there was almost complete agalactia. The placenta was retained in each case, but there was no other evidence of ill health in the cows. Any other cause of abortion of bacterial, viral, fungal, or toxic origin was ruled out, on the basis of laboratory and field investigations.

III.4.2 Experimental animal studies

This topic has been reviewed by Ainsworth & Austwick (1959) and Mantle (1977c). It appears that most reports of field cases and experimental animal studies on ergotism do not contain any information on the contents of individual ergolines in the ergot associated with disease manifestations in animals.

III.4.2.1 Cattle

Lameness, sometimes leading to gangrene, is a common symptom observed in cattle when the feed contains more than 10 g ergot/kg. The symptoms are more pronounced when the animals are kept outside under cold weather conditions (Mantle, 1977c).

Four out of 6 animals administered ergotamine tartrate, orally, at 1 mg/kg body weight per day, died within 10 days (Woods et al., 1966). The animals became acutely ill within 1–2 days, the principal signs being anorexia, hyperventilation, cold extremities, salivation, and, occasionally, tongue necrosis. Post-mortem examination of the most seriously affected animals revealed extensive intestinal inflammation.

III.4.2.2 Sheep

Four lambs were administered aqueous suspensions of milled ergot (from *C. purpurea*) through a stomach tube, over a 2-month period (Loken, 1984). The doses ranged from 0.12 to 0.75 g sclerotia/kg body weight. The sclerotia contained approximately 4 g ergolines/kg, composed of ergotamine (15%), ergosine (35%), and ergocristine (5% each). One lamb, dosed with 0.12 g sclerotia/kg body weight, was kept indoors at 15–17 °C and did not develop any symptoms. The other 3 animals, given higher doses and kept outdoors, became ill after 2–6 days, with signs that included dullness, inappetence, high pulse rate, diarrhoea, edema of the hind legs and tail, and lameness. Post-mortem findings included inflammation and necrosis of the forestomach and intestinal mucosa.

III.4.2.3 Poultry

Leghorn chickens were fed diets containing ergotamine tartrate at levels of up to 800 mg/kg in 7 to 10-day trials as well as in a 51-day trial (Young & Marquardt, 1982). In the short-term trials, only the highest level (800 mg/kg) had an effect on performance, in terms of a slight decrease in growth rate and a slight increase in feed consumption. At the 250 mg/kg level, toe necrosis was observed, as well as cardiomegaly. There were no pathological effects on the brain, liver, or muscle tissues, even at the highest level. In the long-term study (51 days), effects were similar to those observed in the 7 to 10-day trials. No residues of ergotamine were detected in tissues.

III.4.2.4 Swine

Ergot (from *C. purpurea*) containing 0.3% ergolines (composition: ergotamine and ergosine) was used in a feeding study on pigs (Mantle, 1977c). A diet containing ergot at 40 g/kg was well tolerated, and the only effect at 100 g ergot/kg diet was depression of the growth rate. Agalactia in the sow was observed after feeding with rations containing ergot from *C. purpurea* as well as from *C. fusiformis*.

III.4.2.5 Primates

Male rhesus monkeys in groups of 2–4 animals were dosed with ergot from *C. fusiformis*, either as part of the diet, or as an ergoline extract administered orally or intraperitoneally (Bhat & Roy, 1976). The ergoline extract contained agroclavine (major components: elymoclavine, chanoclavine, penniclavine, and setoclavine). The period of treatment lasted from 2 days to one month. There were no effects on the animals, with the exception of those injected ip with 5.44–11.1 mg total ergoline/kg body weight, who developed signs within 10 min including drowsiness, hyperexcitation, redness of face, and loss of response to thermal and tactile stimuli in the hind limbs and tail. The animals recovered spontaneously in about 60 min. Animals administered a total of 10 mg ergolines/kg body weight, orally, showed symptoms of hyperexcitation, but these were far less severe than those after intraperitoneal injection. It was concluded that the signs in monkeys, in particular hyperexcitation, are different from those observed in human beings after ingestion of ergot in pearl millet.

III.5 EFFECTS ON MAN

The history of ergotism in man, following ingestion of ergot from *C. purpurea*, was reviewed by Barger (1931). Numerous epidemics in Europe occurred between the 9th and the 18th century. Two types of disease were noted: gangrenous and convulsive ergotism. In the first type, the affected part (arm or leg) shrank, became mummified and dry, and the gangrene gradually spread upwards. In convulsive ergotism, the whole body was attacked by general convulsion, which returned at intervals of a few days. The latest outbreaks of ergotism in Europe occurred in 1926–28 in the United Kingdom and the USSR. A suspected episode in France in 1951 turned out to be due to a different toxic substance (Gadiou, 1965).

III.5.1 Ergometrine-related outbreaks

In 1978, an epidemic was reported in Ethiopia (Demeke et al., 1979; King, 1979; Pokrovskij & Tutelyan, 1982). The episode occurred in the Wollo region, following two years of drought. During this time, the locally grown barley, the staple food, had become dominated by wild oats heavily contaminated with *C. purpurea* sclerotia. The grain consisted of 70% wild oats, 12% barley, and 0.75% ergot; ergometrine was detected in the sclerotia by thin layer chromatography. A total of 93 cases of ergotism was reported during the spring of 1978. The male: female ratio was 2.5:1; more than 80% of affected persons were between five and 34 years of age. In addition to the 93 cases, 47 deaths were reported as having been due to ergotism. Examination of 44 patients out of the 93 registered revealed ongoing dry gangrene of the whole or part of one or more limbs (7.5%), feeble or absent peripheral pulses (36.4%), swelling of limbs (11.2 %), desquamation of the skin (12.8%), and loss of one or more limbs (21.5%). It was noted that 88% of patients had involvement of the lower extremities. The most common general symptoms were weakness (78.5%), formication (15%), burning sensation (14.3%), nausea (7.2%), vomiting (5.6%), and diarrhoea (6.8%). In addition, 50–60 infants and young children died

from starvation due to failure of the mothers to lactate. This may have been related to the effect of ergot on lactation (Demeke et al., 1979). No autopsies were performed, and thus there is no information on pathological changes in the viscera.

III.5.2 Clavine-related outbreaks

Intoxication following ingestion of ergot from *C. fusiformis* in bajra or pearl millet has been reported from India. Symptoms included nausea, vomiting, and giddiness. Several outbreaks have been observed since 1958, when the first report was published; the latest occurred in the autumn of 1975 in the state of Rajasthan (Krishnamachari & Bhat, 1976). In 21 villages surveyed, 78 persons belonging to 14 households developed symptoms, characterized by nausea, repeated vomiting, and giddiness, followed by drowsiness and prolonged sleepiness, extending sometimes to over 24–48 h. There were no signs or symptoms suggesting vaso-occlusion. The disease generally developed 1–2 h following a single meal. Domestic camels, offered the contaminated grain as feed, also developed sleepiness and signs suggesting abdominal discomfort. The pearl millet from affected villages contained 15–174 g ergot/kg, resulting in a contamination of the grain with 15–199 mg total ergolines/kg. The individual ergolines were identified as agroclavine, elymoclavine, chanoclavine, penniclavine, and setoclavine. Pearl millet from villages with no cases of intoxication contained 1–38 g ergot/kg with a total ergoline content of 15–26 mg/kg. Since there were no deaths, no information on pathological changes is available. The number of households studied was too small for no-effect levels to be calculated, but the authors suggested that the intake of 28 μg total ergolines/kg body weight would be non-toxic.

III.6 EVALUATION OF THE HUMAN HEALTH RISKS

Human exposure to low levels of ergolines appears to be widespread. Available data from the recent outbreaks in Ethiopia and India indicate that the *C. purpurea* alkaloids (ergotamine group) produced more severe effects, including gangrene of the legs and death, than the alkaloids of *C. fusiformis* (clavine group), which caused gastrointestinal symptoms without a fatal outcome. It is not known whether such differences can be accounted for by differences in the alkaloid content of the fungal species, in the toxicological or toxicometric properties of the alkaloids, or in the levels of intake by different types of populations.

Only low levels of ergolines remain in prepared foods as cleaning and milling processes remove the sclerotia; baking or other heat processing also destroys most alkaloids of the ergotamin group.

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RESUME ET RECOMMANDATIONS EN VUE DE RECHERCHES FUTURES

1. Ochratoxine A

1.1 Etat naturel

Les ochratoxines sont produites par plusieurs espèces de champignons appartenant au genre *Aspergillus* et *Penicillium*. Il s'agit d'espèces ubiquistes, aussi le risque de contamination des denrées alimentaires destinées à l'homme et aux animaux est-il omniprésent. Le principal composé, l'ochratoxine A se rencontre en Australie ainsi que dans certains pays d'Europe et d'Amérique du Nord. La production d'ochratoxine par les espèces du genre *Aspergillus* semble limitée aux environnements très chauds et humides alors que dans le cas de *Penicillium*, au moins certaines espèces peuvent produire de l'ochratoxine à des températures n'excédant pas 5 °C.

Ce sont les céréales qui sont le plus fréquemment contaminées par l'ochratoxine A et à un moindre degré certaines fèves (café, soja, cacao). L'ochratoxine B est très rare.

1.2 Méthodes d'analyse

On a mis au point des méthodes d'analyse permettant l'identification et le dosage de l'ochratoxine à des concentrations de l'ordre de 1 µg/kg.

1.3 Métabolisme

On trouve des résidus d'ochratoxine A intacte dans le sang, les reins, le foie et les muscles de porcs à l'abattoir ainsi que dans les muscles de poules et de poulets. En revanche on ne trouve généralement pas de résidus chez les ruminants. *In vitro*, l'ochratoxine A se fixe très solidement à l'albumine sérique bovine, porcine et humaine. Des études expérimentales sur des porcins et des poules ont montré que c'est au niveau des reins

que les concentrations d'ochratoxine A sont les plus élevées. Chez le porc, le rat et l'homme, il pourrait y avoir détoxication par hydroxylation au niveau des microsomes. L'expérience montre qu'on peut encore identifier des résidus dans les reins de porc un mois après la fin de l'exposition.

1.4 Effets sur les animaux

On a signalé des cas d'ochratoxicose chez des animaux d'élevage (porcs et volaille) dans plusieurs pays d'Europe, la manifestation essentielle étant une néphropathie chronique. Les lésions se présentent sous la forme d'une atrophie tubulaire, d'une fibrose interstitielle et à un stade plus avancé, d'une hyalinisation des glomérules. Au Canada, on a également trouvé de l'ochratoxine A dans du sang de porc recueilli à l'abattoir. Des effets néphrotoxiques ont été relevés chez toutes les espèces d'animaux à estomac simple étudiées jusqu'ici, même aux doses les plus faibles qui aient été expérimentées (200 mg/kg de nourriture chez les rats et les porcs).

Des effets tératogènes ont été observés chez des souris exposées par voie orale à 3 mg d'ochratoxine A par kg de poids corporel. On a observé une résorption des foetus chez des rats à partir de 0,75 mg/kg de poids corporel, le produit étant administré par voie orale. Ces effets tératogènes, qui chez le rat sont accrus par un régime alimentaire pauvre en protéines, ont été également observés chez des hamsters.

Les épreuves à court terme n'ont pas permis de mettre en évidence d'activité mutagène (bactéries et levures). Administré par voie orale à des rats, le produit a provoqué des ruptures monocaténaires dans l'ADN des tissus du rein et du foie. L'ochratoxine a provoqué l'apparition de cancers du rein chez des souris mâles et des rats des deux sexes qui recevaient le produit par voie orale. La formation de carcinomes hépatocellulaires n'a été signalée que chez une souche de souris et pas du tout chez le rat.

L'ochratoxine A est un inhibiteur de la synthèse protéique et de la tRNA-synthétase chez les microorganismes et dans les cellules hépatomateuses; elle inhibe également le mARN chez le rat.

L'ochratoxine A peut inhiber la migration des macrophages. Chez la souris, une dose de 0,005 µg/kg de poids corporel a aboli la réponse immunitaire aux érythrocytes de mouton; toutefois d'autres résultats, contradictoires, ont été obtenus.

L'ochratoxine A s'est révélée cancérogène pour l'épithelium tubulaire rénal chez des souris mâles et des rats des deux sexes.

1.5 Effets sur l'homme

L'exposition humaine, qui ressort de la présence d'ochratoxine A dans les aliments, le sang et le lait humains, a été observée dans divers pays d'Europe. Selon les données épidémiologiques disponibles, la néphropathie des Balkans peut être attribuée à la consommation de denrées alimentaires contaminées par cette toxine.

On a mis en évidence une relation tout à fait significative entre la néphropathie des Balkans et les tumeurs des voies urinaires, en particulier des tumeurs pyéliques et uretérales. Toutefois on n'a pas publié de données qui établissent une implication directe de l'ochratoxine A dans l'étiologie de ces tumeurs.

2. Trichothécènes

2.1 Etat naturel

On connaît aujourd'hui 148 trichothécènes qui se caractérisent sur le plan chimique par la présence d'une structure de base commune, le système tétracyclique du scirpénol. Ces composés sont principalement secrétés par des moisissures appartenant au genre *Fusarium*, encore que d'autres genres notamment *Trichoderma*, *Trichothecium*, *Myrothecium* et *Stachybotrys* produisent également des métabolites considérés désormais comme des trichothécènes. Quelques-uns seulement de ces trichothécènes contaminent les denrées alimentaires destinées à l'homme ou aux animaux, en particulier : le désoxyvalénol (DON), le nivalénol (NIV), le disacétoxyscirpenol (DAS), ainsi que la toxine T-2 et, plus rarement, certains dérivés (3-Ac-DON, 15-Ac-DON, fusarenone-X et toxine HT-2). Parmi ces substances, c'est le DON qui est de loin la plus fréquemment

présente dans les denrées alimentaires destinées à l'homme et aux animaux; à côté de quantités plus faibles de NIV. Certains trichothécènes macrocycliques comme les satratoxines G et H et les verrucarines, se rencontrent de temps à autre dans les fourrages (paille, foin) mais il n'a pas été fait état de leur présence dans les produits alimentaires.

Les enquêtes sur la présence de trichothécènes ont révélé que le DON était présent dans le monde entier, essentiellement dans les céréales telles que le froment et le maïs à des concentrations pouvant parfois atteindre 92 mg/kg, encore que les teneurs moyennes soient beaucoup plus faibles et varient selon la denrée. Des rapports isolés font état de la présence de DON dans de l'orge, des mélanges alimentaires pour aminaux, des pommes de terre, etc. Le NIV, dont la présence n'est normalement pas signalée dans des céréales au Canada ou aux Etats-Unis se rencontre en revanche fréquemment au côté du DON dans les céréales originaires d'Asie ou d'Europe; la concentration la plus forte enregistrée jusqu'ici est de 37,9 mg/kg. On a signalé ça et là la présence de toxine T-2 et de DAS à des concentrations beaucoup plus faibles.

Des études portant sur les divers traitements subis par les produits alimentaires et notamment la mouture, montrent que, entre la céréale brute et le produit définitif, il n'y a guère de diminution des teneurs en DON. De même la panification ne parvient pas à détruire le DON. En général, les aliments destinés à l'homme que l'on trouve dans le commerce ne contiennent que rarement des quantités décelables de DON et de NIV.

2.2 *Méthodes d'analyse*

On dispose, pour le dosage des quatre toxines les plus fréquemment rencontrées (DON, toxine T.2, DAS et NIV), des méthodes d'analyse basées sur la chromatographie en couche mince, la chromatographie en phase gazeuse ou la chromatographie liquide à haute performance ainsi que sur des réactions immunologiques; les limites de détection se situent en-dessous de 1 µg/g. Plusieurs de ces méthodes ont fait l'objet d'une expérimentation collective. En outre, certaines méthodes utilisées en recherche comme la chromatographie gazeuse ou

liquide associée à la spectrographie de masse peuvent être utilisées pour confirmer l'identité des substances.

2.3 Métabolisme

On a procédé à des études métaboliques, essentiellement de la toxine T-2, mais dans quelques rares cas seulement du DON, chez l'animal. Ces trichothécènes sont rapidement absorbés au niveau des voies digestives mais l'on ne dispose pas de données quantitatives. Les toxines se répartissent de façon uniforme sans accumulation marquée au niveau d'un organe ou d'un tissu particulier. Elles sont métabolisées en produits moins toxiques par hydrolyse, hydroxylation, désépoxydation et glucuronidation. La toxin T-2 et le DON sont rapidement éliminés par voie fécale et urinaire.

Par exemple, une dose de toxine T-2 administrée par voie orale à des bovins a été éliminée presque à hauteur de 100 % dans les heures suivant l'administration; des poulets ont éliminé 80 % de la substance dans les 48 heures suivant l'administration. Chez le rat, 25 % d'une dose de DON ont été éliminés dans les urines et 65 % dans les matières fécales 96 heures après l'administration. Chez la poule pondeuse et la vache laitière on a constaté que moins de 1 % de la dose de toxine T-2 (et de ses métabolites) qui leur avait été administrée se retrouvaient dans les oeufs et le lait. Après administration par voie orale de toxine T-2 à des poulets, on a constaté que les résidus présents dans la viande 24 heures plus tard représentaient moins de 2 % de la dose initiale.

2.4 Effets sur les animaux

C'est principalement par l'ingestion de fourrage contaminé que les animaux sont exposés aux trichothécènes. La toxine T-2 et le DAS qui chez les animaux de laboratoire sont les plus actifs des trichothécènes couramment cités comme contaminants des denrées destinées aux animaux (toxine T-2, DAS, NIV et DON), provoquent des réactions toxiques analogues. Le NIV est moins actif dans certains systèmes que les deux précédents composés et le DON est le moins toxique des quatre. (Cette activité toxique peut s'évaluer au moyen de la DL_{50} pour la souris, par

exemple dans le cas de la toxine T-2 elle est égale à 10,5 mg/kg de poids corporel et dans le cas du DON à 46,0 mg/kg).

Les trichothécènes les plus actifs, tels que la toxine T-2 et le DAS produisent des effets généraux aigus lorsqu'ils sont administrés expérimentalement à des rongeurs, des porcs et des bovins par voie orale, parentérale ou respiratoire (porc, souris). Ces trichothécènes produisent une épithélionécrose par contact (une dose de 0,2 μg par touche dans le cas de la toxine T-2). Avec les autres trichothécènes, il faut des doses plus élevées pour obtenir un effet irritant (dans le cas du NIV, 10 ug par touche). Les trichothécènes cytotoxiques comme la toxine T-2 ont une action nécrosante sur l'épithélium des cryptes intestinales et sur les tissus lymphoïdes et hematopoïétiques après exposition par voie orale, parentérale ou respiratoire. Après exposition à la toxine T-2 et au DAS on observe des anomalies hematologiques et des troubles de l'hémostase. Dans les cas graves, la toxicose peut entraîner une pancytopénie. Des études portant sur la toxine T-2, le DON et le DAS ont mis en évidence la suppression de l'immunité à médiation cellulaire et de l'immunité humorale et on a observé une réduction de la concentration des immunoglobulines ainsi qu'une dépression de l'activité phagocytaire des macrophages et des neutrophiles. Des études sur animaux de laboratoire ont montré que l'effet immunodépresseur de trichothécènes tels que la toxine T-2, le DAS et le DON entraînait une moindre résistance aux infections secondaires par des bactéries (Mycobactérie, *Listeria monocytogenes*) des levures (*Cryptococcus neoformans*) et des virus (virus de l'herpes simplex).

Il a été indiqué qu'après injection intrapéritonéale, la toxine T-2 était tératogène pour la souris (cette voie d'administration n'est pas courante dans les études de tératogénicité). Le DON est tératogène pour la souris après intubation gastrique mais ne l'est pas chez le rat lorsque la toxine est administrée dans la nourriture de l'animal. Le NIV ne s'est pas révélé tératogène pour la souris. La recherche du pouvoir mutagène de la toxine T-2, du DAS et du DON par une épreuve du type Ames n'a pas donné de résultat positif. La toxine T-2 présente dans certaines épreuves une faible activité clastogène. D'après les études de toxicité à long terme qui ont été publiées, rien n'indique que la toxine T-2, la

fusarénone-X et le NIV ne soient tumorigènes chez l'animal. Aucune étude de toxicité à long terme n'a été publiée sur le DON.

Les trichothécènes sont toxiques pour les cellules à forte activité mitotique telles que les cellules de l'épithélium des cryptes intestinales et les cellules hematopoïétiques. Cette cytotoxicité proviendrait, soit d'une perturbation de la synthèse des protéines par une fixation des composés aux ribosomes des cellules eucaryotes, soit d'une dysfonction des membranes cellulaires. L'inhibition de la synthèse protéique serait due à l'induction de protéines régulatrices labiles telles que l'IL-2 dans les immunocytes. A concentrations extrêmement faibles, les trichothécènes perturbent le transport des petites molécules à travers les membranes cellulaires.

2.5 *Effets sur l'homme*

L'ingestion de produits alimentaires contaminés d'origine végétale constitue la principale voie d'exposition aux trichothécènes mais d'autres voies ont été signalées à l'occasion, par exemple un contact cutané accidentel chez des chercheurs de laboratoire ou l'inhalation de trichothécènes présents dans des poussières aéroportées.

On n'a décrit que peu de cas de maladies attribuables à une exposition aux trichothécènes, sans d'ailleurs que la responsabilité de ces produits soit établie. Toutefois, dans les deux flambées évoquées ci-dessous, il y a lieu de penser que leur responsabilité est en cause.

Une des flambées, qui s'est produite en Chine, a été attribuée à la consommation de blé moisi contenant 1,0 à 40,0 mg de DON par kg. La maladie se caractérisait par des symptômes gastro-intestinaux. Aucun décès n'a été à déplorer. Les porcs et les poulets qui avaient mangé les restes de céréales ont également été affectés.

Une flambée du même genre a été signalée en Inde et attribuée à la consommation de pain fabriqué à l'aide de blé contaminé. La maladie se caractérisait par des symptômes gastro-intestinaux et une irritation de la gorge qui apparaissaient 15 minutes à une

heure après l'ingestion du pain. On a décelé les mycotoxines suivantes dans des échantillons de farine raffinée utilisée pour la préparation du pain : DON (0,35-8,3 mg/kg), acétyldésoxynivalénol (0,64-2,49 mg/kg), NIV (0,03-0,1 mg/kg) et toxine T-2 (0,5-0,8 mg/kg). Toutefois, l'identité de ces trichothécènes n'a pas été confirmée. La présence de DON et de NIV à côté de la toxine T-2 est inhabituelle.

Deux maladies d'intérêt historique, une aleucie alimentaire d'origine toxique en URSS et une toxicose due à du blé moisi au Japon et en Corée ont été attribuées à la consommation de céréales contaminées par des moisissures du genre *Fusarium*. Depuis lors, on a isolé en laboratoire, dans des cultures de *Fusarium* isolés des céréales en cause, un certain nombre de trichothécènes. Il n'avait pas été possible, au moment où ces maladies se sont déclarées, d'effectuer des recherches pour tenter de corréler l'aleucie toxique et la toxicose due au blé moisi à une exposition à des trichothécènes car on ne connaissait pas les toxines en question.

3. Ergot

3.1 Etat naturel

Ergot est le nom que l'on donne aux sclérotes de certaines espèces de champignons appartenant au genre cClavicepsa. Ces sclérotes contiennent des alcaloïdes biologiquement actifs qui peuvent être à l'origine de toxicoses chez les personnes ou les animaux qui consomment des denrées contaminées.

Les alcaloïdes de l'ergot produisent deux types de maladies selon leur nature, qui dépend du champignon en cause (*C. purpurea*, *C. fusiformis*). L'ergotisme, dû à l'ergotamine et à l'ergocristine produites par *C. purpurea* se caractérise principalement par une gangrène des extrémités et des symptômes gastro-intestinaux. Quand à l'intoxication provoquée par du millet contaminé par *C. fusiformis*, elle se caractérise principalement par des symptômes digestifs et elle est due aux clavines. Aucun des signes ou symptômes observés ne sont révélateurs d'une oblitération vasculaire.

3.2 Méthodes d'analyse

Les alcaloïdes de l'ergot (ergolines) sont des dérivés de l'acide lysergique. Ils ont une activité biologique variable selon leur nature. Le dosage des alcaloïdes de *C. purpurea* a été effectué par chromatographie liquide à haute performance avec détection par fluorescence. On peut déterminer des concentrations de 0,2 µg d'ergolines par litre de plasma humain. La détermination de l'ergotamine et de l'ergocristine peut s'effectuer de façon très spécifique par titrage radio-immunologique à des concentrations respectives de 3,5 et 0,8 picomoles.

3.3 Effets sur les animaux

Des flambées d'avortements chez des bovins ont pu être attribuées à l'ingestion d'ergoline, essentiellement de l'ergotamine et de l'ergotaminine. Des moutons à qui l'on avait administré de l'ergotamine par voie orale sont tombés rapidement malades et présentaient une inflammation intestinale. Chez des volailles, des porcs et des primates exposés par voie orale on a observé des effets légers. Le groupe de travail ne disposait d'aucune donnée sur la mutagénicité, la tératogénicité et la cancérogénicité des ergolines.

3.4 Effets sur l'homme

L'homme peut être exposé aux ergolines par la consommation de céréales ergotées. Dans la plupart des études toxicologiques qui ont été effectuées, on n'a pas procédé à l'identification précise des alcaloïdes en cause. Les données qui ont été publiées au sujet d'une seule enquête effectuée en Suisse sur des céréales et des produits céréaliers indiquent que la consommation quotidienne totale d'ergolines se situe à environ 1,5 ug par personne, certaines denrées en contenant jusqu'à 140 µg/kg. La panification réduit de 25 à 100 % la teneur en ergolines des farines contaminées.

En Ethiopie, une flambée d'ergotisme s'est produite en 1978 à la suite de l'exposition à des ergolines provenant de sclérotes de ce *C. purpurea*. Les céréales comptaient jusqu'à 0,75 % d'ergot;

on a relevé la présence d'ergométrine. Parmi les symptômes observés figuraient une gangrène sèche ayant entraîné la perte d'un ou plusieurs membres (29 % des cas), un pouls périphérique faible ou absent (36 %) et une desquamation. Des symptômes digestifs n'ont été observés que dans quelques cas. Chez 88 % des malades, les lésions intéressaient les membres inférieurs.

En Inde, plusieurs flambées se sont déclarées depuis 1958 à la suite de la consommation de millet contaminé par *C. fusiformis*. Ces symptômes consistaient en nausées, vomissements et vertiges. Les symptômes toxiques étaient dus à la présence d'ergolines à des concentrations de 15 à 26 mg/kg. Aucune autopsie n'ayant été effectuée, on ne dispose d'aucune information sur les effets pathologiques au niveau des viscères.

4. Evaluation des risques pour la santé humaine

4.1 Ochratoxine A

L'exposition humaine, observée dans plusieurs pays d'Europe est objectivée par la présence d'ochratoxine A dans les denrées alimentaires et le sang. Le groupe de travail n'a pas connaissance de tentatives qui ont effectuées dans d'autres régions du monde pour déceler la présence d'ochratoxine A dans le sang humain.

En s'appuyant sur l'étude de la maladie – naturelle ou provoquée par administration d'ochratoxine A – on a pu établir le rôle étiologique de l'ochratoxine A dans la néphropathie porcine. A partir de ce modèle, on a pu émettre l'hypothèse que la néphropathie endémique des Balkans était due à une exposition à cette toxine. Les données immunologiques disponibles montrent que cette affection serait attribuable à la consommation de denrées alimentaires contaminées par de l'ochratoxine A. Depuis la publication en 1980 du No 11 des Critères d'hygiène de l'environnement, des études épidémiologiques sur la concentration de l'ochratoxine A dans le sang humain dans les régions touchées et non touchées ont conforté l'hypothèse d'une relation entre la néphropathie balkanique et l'exposition à l'ochratoxine A.

Ainsi, on a montré que les habitants des régions d'endémie présentaient plus fréquemment de l'ochratoxine A dans leur sang et à des concentrations plus élevées. Cependant, ces études rétrospectives ne fournissent que des présomptions sur lesquelles on ne peut établir l'existence d'une relation causale directe. On ne peut cependant pas l'exclure du fait de la longue période de latence entre l'exposition et l'apparition des symptômes.

On a montré que l'ochratoxine A exerçait, chez les souris mâles et les rats des deux sexes, des effets cancérogènes sur l'épithélium des tubules rénaux. Il existe une relation tout à fait significative entre la néphropathie balkanique et la présence de tumeurs des voies urinaires, notamment de tumeurs pyéliques et uretérales. Toutefois aucune donnée n'a été publiée qui établissent la responsabilité directe de l'ochratoxine A dans l'étiologie de ces tumeurs.

4.2 Trichothécènes

Sur la base des données dont disposait le groupe de travail, il est possible d'établir une relation entre l'exposition aux trichothécènes et certains épisodes toxiques chez l'homme. Si l'on se réfère aux quelques données disponibles, ce sont le DON et le NIV qui sont les plus fréquemment cités dans les différents cas d'exposition humaine. Dans le cas des flambées d'aleucie toxique alimentaire et de toxicose par ingestion de céréales moisies, on ne peut exclure la responsabilité des trichothécènes. L'exposition se produit par ingestion de denrées contaminées, principalement des céréales. Les différents traitements qu'elles subissent, notamment la mouture et la panification ne permettent pas d'éliminer le DON, le NIV, ni la toxine T-2. L'existence d'une exposition par voie respiratoire est très peu documentée mais c'est une possibilité qu'on ne peut totalement écarter.

Parmi les tricothécènes qui se trouvent à l'état naturel dans les aliments, c'est la toxine T-2 qui est la plus active, suivie du DAS and du NIV; les études de toxicité aiguë ont montré que le DON était la substance la moins toxique. Chez l'animal d'expérience, la toxine T-2 et le DAS déterminent des symptômes généraux aigus, avec nécrose des tissus épithéliaux et suppression de

l'hematopoïèse. Lors des récentes flambées d'intoxications, il n'a été fait état que des symptômes digestifs.

Le DON est tératogène pour la souris mais pas pour le rat. Selon les études de toxicité chroniques qui ont été publiées, le NIV et la toxine T-2 ne sont pas tumorigènes chez l'animal. Aucune étude de cancérogénicité à long terme n'a été publiée sur le DON. Certains trichothécènes, tels que la toxine T-2 et le DON ont une action immunosuppressive chez l'animal et produisent des modifications de l'immunité à médiation cellulaire et de l'immunité humorale. En revanche, rien n'indique l'existence de tels effets chez l'homme.

On est mal informé sur les cas d'intoxication humaine due à une exposition aux trichothécènes, cas qui sont en nombre limité. Les symptômes observés consistent en troubles digestifs et irritation de la gorge; ils apparaissent peu après l'ingestion de denrées alimentaires contaminées par des trichothécènes. A l'heure actuelle, on ne connaît pas de cas de cancer humain attribuable aux trichothécènes. Le groupe de travail ne disposait d'aucune publication faisant état d'infections secondaires d'origine bactérienne, fongique ou virale chez l'homme, à la suite d'une exposition aux trichothécènes comme on en a observé chez l'animal d'expérience. Il semble que l'on n'ait pas effectué d'études appropriées sur ce point.

4.3 Ergot

Il semble que l'exposition humaine à de faibles concentrations d'ergolines soit très répandue. Les données relatives aux flambées qui se sont récemment déclarées en Ethiopie et en Inde montrent que les alcaloïdes de *C. purpurea* (groupe de l'ergotamine) produisent les effets les plus graves, notamment une gangrène des membres inférieurs pouvant entraîner la mort, effets qui sont plus graves que ceux des alcaloïdes de *C. fusiformis* (groupe de la clavine) qui entraînent des symptômes digestifs sans issue fatale. On ignore si ces différences s'expliquent par la teneur des différentes espèces de champignons en alcaloïdes, par les propriétés toxicologiques de ces substances ou par des différences dans les quantités ingérées par les diverses populations.

Après nettoyage et mouture qui éliminent les sclérotes, il ne subsiste dans les produits alimentaires que de faibles quantités d'ergolines. La panification ou d'autres traitements thermiques détruisent également la plupart des alcaloïdes du groupe de l'ergotamine.

5. Recommandations en vue de recherches futures

5.1 Recommandations generales

Il conviendrait de créer un réseau de centres de référence pour aider les Etats Membres à confirmer l'identité des mycotoxines présentes dans les tissus humains et les denrées destinées à la consommation humaine. Ces centres devraient également fournir sur demande des échantillons de référence de mycotoxines afin de faciliter la comparabilité des résultats d'analyse obtenus dans les différentes régions du monde.

5.2 Ochratoxine A

a) Etudes épidémiologiques de grande ampleur à caractère rétrospectif et études localisées à caractère prospectif sur l'association entre l'ochratoxine A et la néphropathie endémique des Balkans ainsi que les tumeurs des voies urinaires : ces études devraient être menées dans la péninsule de Balkans et la Région méditerranéenne.

b) Recherche et dosage de l'ochratoxine A dans le sang de malades porteurs de tumeurs des voies urinaires, à l'extérieur de la péninsule des Balkans.

c) Recherche de sources d'exposition à l'ochratoxine A, par analyse du sang, dans les pays situés en dehors de la péninsule des Balkans.

d) Elucidation du mécanisme à l'origine des différences entre les sexes qui ont été relevées dans les anomalies rénales, néoplasiques et non-néoplasiques produites par l'ochratoxine A chez l'animal d'expérience.

e) Etudes de grande envergure sur la teneur en ochratoxine A des denrées alimentaires dans les différentes régions du monde.

Ce type d'enquêtes est particulièrement important dans les régions où l'on observe une forte incidence de tumeurs des voies urinaires, notamment rénales, ou de néphropathies.

5.3 Trichothécènes

a) Il conviendrait d'effectuer des études longitudinales dans les régions de l'Inde et de la République populaire de Chine où ont été récemment observés des épisodes d'intoxication par les trichothécènes. Il conviendrait de mieux éclaircir la présence inhabituelle de certains trichothécènes dans ces régions.

b) Il faudrait étudier les effets d'une exposition prolongée d'animaux de laboratoire au DON, et notamment les effets cancérogènes. Comme la réaction au DON varie beaucoup selon les espèces, l'espèce qui sera retenue devra être choisie avec soin.

c) Il faudrait étudier plus à fond les infections microbiennes secondaires à une exposition aux trichothécènes chez l'animal d'expérience.

d) Il convient d'étudier l'influence des conditions environnementales, notamment la présence d'insecticides ou autres produits chimiques industriels, sur la production de trichothécènes par des champignons.

e) Il faudrait élucider l'effet des différents modes de préparation des denrées alimentaires sur les trichothécènes.

f) Il conviendrait de mettre au point des espèces végétales qui résistent aux champignons producteurs de trichothécènes, en recourant aux biotechnologies.

g) Il faudrait étudier sur l'animal les effets synergistiques éventuels d'une exposition simultanée aux trichothécènes, aux aflatoxines, à l'ochratoxine A et à d'autres mycotoxines.

h) Il faudrait également déterminer l'apport de trichothécènes d'origine alimentaire chez l'homme.

i) Il faudrait mettre au point des méthodes de criblage des trichothécènes qui soient à la fois rapides et sensibles, et mener des enquêtes dans les régions tempérées du monde pour

rechercher la présence de trichothécènes dans les céréales et les préparations alimentaires.

5.4 *Ergot*

a) Il faudrait mettre au point des méthodes pour l'analyse des agroclavines.

b) Il conviendrait de mettre à la disposition des pays en développement des renseignements sur le repérage des semences contaminées et sur les méthodes de mouture permettant de réduire au minimum les problèmes posés par la présence de l'ergot.

c) Il faudrait effectuer des études épidémiologiques sur les effets éventuels que peut entraîner chez l'homme l'ingestion de faible quantités d'ergolines.

d) Il faudrait effectuer des études pharmacologiques et toxicologiques sur les ergolines, seules ou en association, chez l'animal d'expérience.

e) Il conviendrait de déterminer si les ergolines peuvent se transmettre de la mère à l'enfant par le lait maternel.

RESUMEN Y RECOMENDACIONES PARA ULTERIOR INVESTIGACION

1. Ocratoxina A

1.1 Distribución natural

Las ocratoxinas son producidas por varias especies de los géneros de hongos *Aspergillus* y *Penicillium*. Esos hongos son ubicuos y hay amplias posibilidades de contaminación de alimentos para seres humanos y para animales. La ocratoxina A, la más importante, se ha hallado en toda una serie de países de América del Norte, Australia y Europa. La formación de ocratoxina por las especies del género *Aspergillus* parece estar limitada a condiciones de humedad y temperatura elevadas, mientras que algunas especies, por lo menos, de *Penicillium* pueden producir ocratoxina a temperaturas de sólo 5 °C.

Las mayores incidencias de contaminación con ocratoxina A se han hallado en cereales y, en menor medida, en algunos granos (café, soja, cacao). La ocratoxina B es muy poco frecuente.

1.2 Métodos de análisis

Se han desarrollado técnicas de análisis para identificar la ocratoxina y determinar sus concentraciones en la gama de g/kg.

1.3 Metabolismo

Se han hallado residuos de ocratoxina A no modificada en la sangre, los riñones, el hígado y los músculos de cerdos en los mataderos y en los músculos de gallinas y pollos. Sin embargo, por lo general no se han hallado residuos de ocratoxina A en los rumiantes. El enlace *in vitro* de la ocratoxina A con la seroalbúmina es especialmente importante en el ganado vacuno, el ganado ovino y el ser humano. Estudios experimentales realizados con cerdos y gallinas han demostrado que las concentraciones más altas de ocratoxina A se hallan en los riñones.

La hidroxilación microsómica puede representar una reacción de detoxificación en los cerdos, las ratas y el ser humano. En estudios experimentales, un mes después de terminada la exposición se localizaron aún residuos en riñones de cerdos.

1.4 Efectos en animales

Varios países europeos han notificado en animales de granja (cerdos, aves de corral) casos de ocratoxicosis, cuya principal manifestación era una nefropatía crónica. Entre las lesiones había atrofia tubular, fibrosis intersticial y, en etapas ulteriores, hialinización de los glomérulos. También se ha hallado ocratoxina A en sangre de cerdo recogida en mataderos canadienses. La ocratoxina A ha producido efectos nefrotóxicos en todas las especies de animales provistas de un solo estómago que se han estudiado hasta el momento, incluso con las dosis más bajas con que se experimentó (200 μg/kg de alimentos en ratas y cerdos).

Se observaron efectos teratogénicos en ratones expuestos a dosis de 3 mg/kg de peso corporal administradas por vía oral. En ratas que recibieron por vía oral dosis de 0,75 mg/kg de peso corporal se observó resorción fetal. Los efectos teratogénicos, que en las ratas se acentuaron con una dieta baja en proteínas, se han observado también en hámsters.

No hay datos que demuestren la actividad de la ocratoxina A en pruebas a corto plazo de la mutagenicidad (bacterias y levaduras). Ratas expuestas a ocratoxina A administrada por vía oral mostraron roturas de una sola cadena del ADN en tejidos renales y hepáticos. Ocratoxina A administrada por vía oral provocó neoplasias de células renales en ratones machos y en ratas de uno y otro sexo. Se registraron neoplasias de células hepáticas en sólo una estirpe de ratones y no en la rata.

La ocratoxina A es inhibidora de la síntesis de proteínas y de la sintetasa del ARNt en microorganismos, células de hepatoma y el ARNm renal en la rata.

La ocratoxina A puede inhibir la migración de los macrófagos. En ratones, una dosis de 0,005 μg/kg de peso corporal suprimió

la respuesta inmunitaria a eritrocitos de oveja; sin embargo, se han obtenido también resultados contradictorios.

Se ha demostrado que la ocratoxina A es carcinógena para el epitelio de los túbulos renales en ratones machos y de ratas de uno y otro sexo.

1.5 Efectos en el ser humano

En varios países de Europa se ha observado exposición humana a ocratoxina A, como lo demuestra la presencia de ésta en alimentos y en la sangre y la leche humanas. Los datos epidemiológicos de que se dispone indican que la nefropatía de los Balcanes podría estar relacionada con el consumo de productos alimenticios contaminados por esta toxina.

Se ha observado una relación muy significativa entre la nefropatía de los Balcanes y tumores del tracto urinario, en particular los de la pelvis renal y los uréteres. Sin embargo, no se han publicado datos que demuestren el papel causal de la ocratoxina A en la etiología de esos tumores.

2. Tricotecenos

2.1 Distribución natural

Hasta ahora se conocen 148 tricotecenos, caracterizados químicamente por la presencia del mismo sistema básico de un anillo tetracíclico de scirpenol. Son compuestos producidos principalmente por hongos pertenecientes al género *Fusarium*, aunque otros géneros, entre ellos *Trichoderma, Trichothecium*, *Myrothecium* y *Stachybotrys*, producen también metabolitos ahora reconocidos como tricotecenos. Se ha visto que sólo un pequeño número de los tricotecenos conocidos contaminan los alimentos para seres humanos o para animales, entre ellos el deoxinivalenol (DON), el nivalenol (NIV), el diacetoxiscirpenol (DAS) y la toxina T-2 y, con menos frecuencia, ciertos derivados (3-Ac-DON,15-Ac-DON, fusarenon-X y toxina HT-2). De todos ellos, el que se encuentra más a menudo en alimentos para seres humanos y para animales es el DON, por lo general con cantidades más pequeñas de NIV como co-contaminante. Algunos

tricotecenos macrocíclicos como las satratoxinas G y H y las verrucarinas se observan ocasionalmente en alimentos para animales (paja, heno) pero no se ha notificado su presencia en alimentos para seres humanos.

Estudios sobre la distribución de los tricotecenos han indicado que el DON se encuentra en todo el mundo, sobre todo en cereales como el trigo y el maíz, en concentraciones que pueden llegar hasta 92 mg/kg, aunque las concentraciones medias son considerablemente inferiores y varían según el producto. Hay notificaciones aisladas de la presencia de DON en la cebada, los piensos compuestos, las patatas, etc. Habitualmente los cereales producidos en Canadá y los Estados Unidos de América no contienen NIV pero éste se ha encontrado en los cereales asiáticos y europeos, junto con DON; la mayor concentración de NIV registrada hasta la fecha fue de 37,9 mg/kg. La presencia de toxina T-2 y DAS se ha notificado con escasa frecuencia y en concentraciones muy inferiores.

Estudios sobre la elaboración y la molienda indican que las concentraciones de DON apenas se reducen del cereal al producto acabado. Tampoco se destruye cuando se cuece el cereal. En general, los productos alimenticios para seres humanos que se encuentran en el comercio rara vez contienen concentraciones detectables de DON y NIV.

2.2 *Métodos de análisis*

Existen xistenmétodos de análisis basados en la cromatografía de capa fina, la cromatografía de fase gaseosa, la cromatografía de fase líquida de alto rendimiento y técnicas inmunológicas para la determinación de las cuatro toxinas más frecuentes (DON, toxina T-2, DAS y NIV) con límites de detección inferiores a 1 μg/g. Algunos de esos métodos se han ensayado en colaboración. Además, la identidad puede confirmarse mediante métodos de investigación como cromatografía de fase gaseosa/espectografía de masas y cromatografía de fase líquida/espectografía de masas.

2.3 *Metabolismo*

Se han realizado estudios metabólicos con animales, sobre todo administrando toxina T-2 y en unos pocos casos DON. Estos tricotecenos se absorben con rapidez por el tubo digestivo, aunque no se dispone de datos cuantitativos. Las toxinas se distribuyen bastante uniformemente, sin marcada acumulación en ningún órgano o tejido determinado. Los tricotecenos se transforman en metabolitos menos tóxicos por reacciones como hidrólisis, hidroxilación, de-epoxidación y glucoronidación. Tricotecenos como la toxina T-2 y el DON se eliminan rápidamente en las heces y la orina. Por ejemplo, casi el 100% de una dosis de toxina T-2 administrada a ganado por vía oral se eliminaba en unas horas; en pollos, alrededor del 80% se había eliminado a las 48 horas. En la rata, 96 horas después de la administración, el 25% del DON se había eliminado en la orina y el 65% en las heces. Los resultados de la transmisión de toxina T-2 en gallinas ponedoras y vacas lactantes indicaron que pasaba a los huevos y a la leche menos del 1% de la dosis administrada de esa toxina y de sus metabolitos. En la carne de pollo, 24 horas después de la administración de toxina T-2 por vía oral, los residuos de ésta y de sus metabolitos eran inferiores al 2% de la dosis.

2.4 *Efectos en animales*

La principal vía de exposición de los animales a tricotecenos es la ingestión de alimentos de origen vegetal. La toxina T-2 y el DAS, los más potentes en los experimentos de laboratorio con animales, de todos los tricotecenos que habitualmente se consideran contaminantes de alimentos para animales (toxina T-2, DAS, NIV y DON), provocan una respuesta tóxica similar. El NIV es menos potente en algunos sistemas que los dos compuestos anteriores y el DON es el menos tóxico de los cuatro (un ejemplo de la potencia es la DL_{50} por vía oral en el ratón: toxina T-2, 10,5 mg/kg de peso corporal y DON, 46,0 mg/kg).

Los tricotecenos más potentes, como la toxina T-2 y el DAS, producen efectos generales agudos cuando se administran experimentalmente a roedores y ganado porcino y bovino por vía

oral o parenteral o por inhalación (cerdo, ratón). Una lesión producida por el contacto con tricotecenos potentes como la toxina T-2 y el DAS es la epitelionecrosis (dosis de 0,2 μg por zona en el caso de la toxina T-2). En cuanto a otros tricotecenos, son necesarias dosis mayores para producir un efecto irritante (NIV, 10 μg por zona). Los tricotecenos citotóxicos, como la toxina T-2, producen necrosis del epitelio de las criptas intestinales y de los tejidos linfoides y hematopoyéticos, tras exposición oral o parenteral o por inhalación. La exposición a tricotecenos citotóxicos como la toxina T-2 y el DAS va seguida de alteraciones hematológicas y coagulopáticas. Las toxicosis graves pueden originar pancitopenia. En estudios con toxina T-2, DON y DAS se ha demostrado la supresión de la inmunidad de base celular y humoral, y entre los efectos observados figuran la reducción de las concentraciones de inmunoglobulinas y la disminución de la actividad fagocítaria de macrófagos y neutrófilos. Los resultados de estudios experimentales con animales han indicado que el efecto inmunodepresor de tricotecenos como la toxina T-2, el DAS y el DON origina una disminución de la resistencia a la infección secundaria por bacterias (micobacterias, *Listeria monocytogenes*), levaduras (*Cryptococcus neoformans*) y virus (virus del herpes simplex).

Se ha comunicado que la toxina T-2 es teratogénica en el ratón, cuando se administra por inyección intraperitoneal (vía de administración poco corriente en los estudios de la teratogenicidad). Se ha comunicado que el DON es teratogénico en los ratones tras intubación gástrica, pero no en las ratas cuando la toxina se administra con los alimentos. El NIV no es teratogénico en el ratón. La toxina T-2, el DAS y el DON no resultaron mutagénicos en una prueba de tipo Ames. En algunas pruebas, se observó una débil actividad clastogénica de la toxina T-2. En los estudios publicados sobre la toxicidad a largo plazo en animales, no se obtuvieron datos que indiquen que la toxina T-2, el fusarenón-X o el NIV sean oncogénicos en los animales. No se han publicado estudios a largo plazo sobre la toxicidad del DON.

Los tricotecenos son tóxicos para las células que se dividen activamente como las del epitelio de las criptas intestinales y las

hematopoyéticas. La citotoxicidad se ha asociado con el trastorno de la síntesis de proteínas debido al enlace de los compuestos con los ribosomas de las células eucarióticas o bien con la disfunción de las membranas celulares. La inhibición de la síntesis de las proteínas se ha relacionado con la inducción de proteínas lábiles y reguladoras como la IL-2 en los inmunocitos. Concentraciones extremadamente bajas de tricotecenos alteran el transporte de pequeñas moléculas en las membranas celulares.

2.5 *Efectos en el ser humano*

La ingestión de alimentos contaminados de origen vegetal es la principal vía de exposición a tricotecenos, pero ocasionalmente se han notificado otras, por ejemplo el contacto accidental con la piel entre los investigadores de laboratorio y la inhalación de tricotecenos transportados por el polvo.

Los casos registrados de enfermedad asociados con la exposición a tricotecenos son escasos y en ninguno de ellos se ha demostrado la relación causal. No obstante, los dos brotes que se describen a continuación parecen indicar su existencia.

En China se registró un brote de enfermedad asociado con el consumo de trigo mohoso que contenía de 1,0 a 40,0 mg de DON/kg. La enfermedad se caracterizó por síntomas gastro-intestinales. No hubo defunciones. Los cerdos y los pollos alimentados con restos de cereales también resultaron afectados.

Un brote análogo se registró en la India, asociado al consumo de pan hecho con trigo contaminado. La enfermedad se caracterizó por síntomas gastrointestinales e irritación de la garganta, que aparecía de 15 minutos a una hora después de la ingestión del pan. En muestras de la harina de trigo refinada utilizada para su preparación se detectaron las siguientes micotoxinas: DON (0,35–8,3 mg/kg), acetildeoxinivalenol (0,64–2,49 mg/kg), NIV (0,03–0,1 mg/kg) y toxina T-2 (0,5–0,8 mg/kg). Sin embargo, no hubo confirmación de la identidad de los tricotecenos detectados. La presencia simultánea de DON y NIV y de toxina T-2 es insólita.

Dos enfermedades de interés histórico, la aleucia tóxica alimentaria (ATA) de la URSS y la toxicosis del trigo mohoso del Japón y Corea se han asociado con el consumo de cereales invadidos por hongos *Fusarium*. Desde entonces se han identificado en condiciones de laboratorio algunos tricotecenos en cultivos de mohos *Fusarium* aislados en cereales encontrados en los incidentes. En la época en que apareció la enfermedad, no pudieron realizarse estudios que relacionaran la ATA o la toxicosis de los cereales mohosos con la exposición a tricotecenos porque las toxinas no eran conocidas.

3. Cornezuelo

3.1 Distribución natural

Cornezuelo es el nombre dado a los esclerocios de especies de hongos pertenecientes al género *Claviceps*. Alcaloides biológicamente activos contenidos en el esclerocio provocan toxicosis cuando éste es consumido por hombres o animales en alimentos contaminados.

Los alcaloides del cornezuelo producen dos modalidades diferentes de enfermedad, según el hongo de que se trate (*C. purpurea*, *C. fusiformis*) y, por lo tanto, los alcaloides producidos. El ergotismo, provocado por los alcaloides ergotamina- ergocristina producidos por *C. purpurea*, se caracteriza sobre todo por gangrena de las extremidades, además de síntomas gastrointestinales. La intoxicación resultante de la ingestión de mijo contaminado por *C. fusiformis* se caracteriza principalmente por síntomas gastrointestinales y está relacionada con los alcaloides de tipo clavina. No hay signos ni síntomas que indiquen la presencia de vaso-oclusión.

3.2 Metodos de analasis

Los alcaloides del cornezuelo (ergolinas) son derivados del ácido lisérgico. Los diversos alcaloides difieren por la importancia de su actividad biológica. La presencia de alcaloides del cornezuelo producidos por *C. purpurea* se ha determinado por cromatografía de fase líquida de alto rendimiento con detección por fluores-

cencia. Pueden medirse concentraciones de 0,2 μg de ergolina por litro de plasma humano. La presencia de ergotamina y ergocristina puede determinarse con gran especificidad mediante pruebas de radioinmunovaloración en concentraciones de 3,5 picomoles y 0,8 picomoles, respectivamente.

3.3 *Efectos en animales*

Las ergolinas, principalmente la ergotamina y la ergotaminina, se han asociado con brotes de abortos en el ganado bovino. Ovejas a las que se administró ergotamina por vía oral enfermaron rápidamente y se observó en ellas inflamación intestinal. Aves de corral, cerdos y primates expuestos por vía oral experimentaron efectos leves. El Grupo Especial no dispuso de datos sobre la mutagenicidad, la teratogenicidad y la carcinogenicidad de las ergolinas.

3.4 *Efectos en el ser humano*

Los cereales infectados por *Claviceps* son fuente de exposición humana a las ergolinas. En la mayor parte de los estudios toxicológicos, no se han identificado los alcaloides específicos. La información resultante de un solo estudio sobre cereales y productos de cereales indica que en Suiza la ingesta total de ergolinas por los seres humanos es de alrededor de 5,1 μg por persona, siendo el contenido de ciertos productos de hasta 140 μg/kg. La cocción reduce las ergolinas presentes en la harina contaminada un 25–100%.

En 1978 hubo en Etiopía un brote de ergotismo, resultante de exposición a ergolinas procedentes de esclerocios de *C. purpurea*. El cereal contenía hasta un 0,75% de cornezuelo; se detectó específicamente ergometrina. Los síntomas fueron gangrena seca, con pérdida de uno o varios miembros (29% de los casos), pulsos periféricos débiles o ausentes (36%) y desescamación de la piel. Sólo hubo síntomas gastrointestinales en unos pocos casos. Trastornos de las extremidades inferiores se registraron en el 88% de los pacientes.

En la India ha habido varios brotes desde 1958 a consecuencia de la ingestión de mijo perlado que contenía cornezuelo de tipo

clavina producido por *C. fusiformis*. Los síntomas comprendían náuseas, vómitos y mareos, y fueron causados por la ingestión de mijo perlado con un contenido de 15 a 26 mg de ergolina/kg.

Como en ninguno de los dos episodios se practicaron autopsias, no se dispone de información sobre los efectos anatomopatológicos en las vísceras humanas.

4. Evaluación de los riesgos para la salud humana

4.1 Ocratoxina A

En diversos países de Europa se ha observado exposición humana a la ocratoxina A, como lo demuestra la presencia de ésta en alimentos y en la sangre. El Grupo Especial no conoce ningún intento de detectar la ocratoxina A en la sangre humana en otras partes del mundo.

El papel causal de la ocratoxina A en la nefropatía porcina se ha demostrado sobre la base de estudios de casos sobre el terreno y de la reproducción de la enfermedad con ocratoxina A. Utilizando el modelo porcino, se ha supuesto que la nefropatía endémica de los Balcanes podría obedecer a exposición a la ocratoxina A. Los datos epidemiológicos de que se dispone indican que esa enfermedad podría estar asociada al consumo de alimentos contaminados por dicha toxina. Desde la publicación de Criterios de salud ambiental 11 en 1979, estudios epidemiológicos sobre concentración de ocratoxina A en la sangre humana en zonas afectadas y no afectadas han proporcionado un argumento más en favor de la relación entre la nefropatía de los Balcanes y la exposición a la ocratoxina A.

Se ha demostrado que tanto la presencia de ocratoxina A en la sangre como sus concentraciones son mayores en las personas que residen en las zonas de endemicidad. No obstante, las pruebas indirectas que proporcionan los mencionados estudios retrospectivos no bastan para demostrar por sí solas la existencia de una relación causal directa. Esta tampoco puede excluirse, dado el largo periodo de latencia entre la exposición y el comienzo de los síntomas.

Se ha demostrado que la ocratoxina A es carcinogénica para el epitelio de los túbulos renales en los ratones machos y en las ratas de uno y otro sexo. Se ha observado una relación muy significativa entre la nefropatía de los Balcanes y los tumores del tracto urinario, en particular de la pelvis renal y los ureteres. Sin embargo, no hay datos publicados que demuestren un papel causal directo de la ocratoxina A en la etiología de esos tumores.

4.2 Tricotecenos

Sobre la base de la información facilitada al Grupo Especial, es posible asociar la exposición a tricotecenos con episodios de enfermedad en los seres humanos. Según los limitados datos disponibles, los tricotecenos que intervienen más frecuentemente en episodios de exposición humana son el DON y el NIV. En los episodios de aleucia tóxica alimentaria y toxicosis por ingestión de cereales mohosos registrados en el pasado, no puede excluirse el posible papel etiológico de los tricotecenos. La exposición se produce por la ingestión de alimentos contaminados, principalmente cereales. La elaboración, la molienda y la cocción no logran la destrucción del DON, el NIV y la toxina T-2. Los datos en favor de la exposición por inhalación son muy limitados, pero no puede excluirse esa posibilidad.

Entre los tricotecenos naturalmente presentes en alimentos, el más potente es la toxina T-2, seguida por el DAS y el NIV; el DON resultó el menos tóxico en los estudios de la toxicidad aguda. En los animales experimentales, la toxina T-2 y el DAS tienen efectos generales agudos, con necrosis de tejidos epiteliales y supresión de la hematopoyesis. En los brotes de enfermedad contemporáneos, sólo se han registrado síntomas gastrointestinales.

Se ha demostrado que el DON es teratogénico en los ratones pero no en las ratas. Según los estudios de la toxicidad crónica publicados, el NIV y la toxina T-2 no son oncogénicos en los animales. No se han publicado estudios sobre la carcinogenicidad a largo plazo del DON. Ciertos tricotecenos, como la toxina T-2 y el DON, tienen una acción inmunosupresora en los animales y han producido alteraciones de la inmunidad, tanto de

base celular como humoral. No hay datos que demuestren la existencia de una acción inmunosupresora en el hombre.

Los casos de enfermedad humana asociados con la exposición a tricotecenos que se han notificado son limitados, tanto por su número como por la información proporcionada. Tras la ingestión de alimentos contaminados con tricotecenos aparecen rápidamente síntomas de trastornos digestivos e irritación de la garganta. En la actualidad no hay nada que demuestre que los tricotecenos causen cáncer en el ser humano. El Grupo Especial no tuvo a su disposición informes sobre infección secundaria por bacterias, hongos o virus en los seres humanos tras la exposición a tricotecenos, como las observadas en estudios experimentales con animales. Al parecer, no se han realizado estudios suficientes para aclarar esa sucesión.

4.3 Cornezuelo

La exposición humana a bajas concentraciones de ergolinas parece estar muy extendida. Los datos de que se dispone sobre los recientes brotes en Etiopía y en la India indican que los alcaloides producidos por *C. purpurea* (grupo de la ergotamina) tienen efectos más graves – entre ellos gangrena de las piernas y muerte – que los alcaloides producidos por *C. fusiformis* (grupo de la clavina), que provocaron síntomas gastrointestinales sin desenlace mortal. No se sabe si esas diferencias corresponden a diferencias en el contenido de alcaloides de las especies de hongos, en las propiedades toxicológicas o toxicométricas de los alcaloides o en las dosis ingeridas por diferentes tipos de poblaciones.

Cuando la limpieza y la molienda eliminan los esclerocios, sólo quedan en los alimentos preparados bajas concentraciones de ergolinas; la cocción y otras aplicaciones de calor destruyen también la mayor parte de los alcaloides del grupo de la ergotamina.

5. Recomendaciones para ulterior investigación

5.1 Recomendaciones generales

Debe establecerse una red de centros de referencia, que ayuden a los Estados Miembros a confirmar la identidad de las micotoxinas halladas en alimentos y tejidos humanos. Esos centros deben proporcionar también muestras de referencia de micotoxinas, cuando se les solicite, a fin de aumentar las posibilidades de comparar los resultados de análisis realizados en distintas partes del mundo.

5.2 Ocratoxina A

a) En los Balcanes y en la región del Mediterráneo deben realizarse estudios epidemiológicos retrospectivos extensos y prospectivos focales sobre la asociación de la ocratoxina A con la nefropatía endémica de tipo balcánico y con tumores del tracto urinario.

b) Fuera de los Balcanes, deben realizarse, en enfermos con tumores del tracto urinario, análisis de sangre para detectar la presencia de ocratoxina A.

c) En países no situados en la zona de los Balcanes, debe aclararse la fuente de la exposición a ocratoxina A, cuando los análisis de sangre humana indiquen que la misma existe.

d) Debe aclararse la manera en que actúa la diferencia de sexo en las enfermedades renales, neoplásicas y no neoplásicas, causadas por la ocratoxina A en animales de experimentación.

e) Son necesarios extensos estudios sobre el contenido de ocratoxina A de los alimentos en distintas partes del mundo. Es especialmente importante realizar estudios de ese tipo en las regiones con una elevada incidencia de tumores del tracto urinario, tumores renales o nefropatías.

5.3 Tricotecenos

a) Deben realizarse estudios de seguimiento en las zonas de la India y de la República Popular China en que ha habido recien-

temente episodios de intoxicación de seres humanos por tricotecenos. Debe aclararse más la modalidad no usual de presencia de tricotecenos en esos episodios.

b) Deben estudiarse los efectos de la exposición a largo plazo de animales de experimentación al DON, incluidos los efectos carcinogénicos. Como la respuesta de distintas especies al DON varía mucho, deben elegirse cuidadosamente las especies utilizadas para el estudio.

c) Debe aclararse más la aparición de infecciones microbianas secundarias en animales de experimentación tras la exposición a tricotecenos.

d) Debe estudiarse la influencia de las condiciones ambientales, incluida la presencia de insecticidas y de otros productos químicos artificiales, en la producción de tricotecenos por los hongos.

e) Deben aclararse los efectos de la elaboración de los alimentos en los tricotecenos.

f) Deben desarrollarse, mediante métodos biotecnológicos, cultivos agrícolas resistentes a la infección por hongos productores de tricotecenos.

g) Deben estudiarse en animales de experimentación los posibles efectos sinérgicos de la exposición combinada a tricotecenos, aflatoxinas, ocratoxina A y otras micotoxinas.

h)Deben realizarse estudios sobre la ingesta de tricotecenos por seres humanos.

i)Deben elaborarse métodos rápidos y sensibles de detección de los tricotecenos y deben realizarse estudios para localizar los tricotecenos en cereales y alimentos tratados en las zonas templadas.

5.4 Cornezuelo

a) Deben hallarse métodos de análisis que permitan detectar la presencia de agroclavinas.

b) Debe proporcionarse a los países en desarrollo información sobre el empleo de procedimientos de detección de semillas patológicas y de molienda para reducir al mínimo los problemas causados por el cornezuelo.

c) Deben realizarse estudios epidemiológicos sobre los posibles efectos de bajas concentraciones de ergolinas en la población humana.

d) Deben efectuarse estudios farmacológicos y toxicológicos en los que se administren a animales de experimentación distintas ergolinas, aisladas y en combinación.

e) Debe aclararse la posible trasmisión de ergolinas a los lactantes a través de la leche materna.

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