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An overview of adverse health effects caused by mycotoxins and bioassays for their detection

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Abstract

The risk of adverse health effects after exposure to moulds and/or their (toxic) metabolites (mycotoxins) present in cereals and cereal products must be determined by carrying out a risk analysis. Part of a risk analysis is hazard characterisation, a study to identify the health effects.

Here, a literature overview is given of the adverse health effects caused by moulds in the genera *Aspergillus*, *Penicillium*, *Fusarium* and *Alternaria* and their mycotoxins. These genera were designated in a previous study as the most important with respect to contamination of cereals and cereal products. The selection of mycotoxins discussed in this report is based on:

1) The occurrence of toxins in cereals and cereal products, as listed in a previous study and
2) Literature of the last ten years, making the acquisition of essential new views on the occurrence and action clear (mainly toxins produced by *Fusarium* and *Alternaria* species) or
3) The reputation of mycotoxins (mainly toxins produced by *Aspergillus* and *Penicillium* species) in causing health problems, so that failure to mention them would lead to incompleteness.

The number and nature of adverse health effects are very diverse. Most have been established in experimental animals. Only a few disorders in humans can be conclusively attributed to mycotoxins. Bioassays can be helpful tools for establishing toxicity of compounds and for research on the exact nature of health effects caused by mycotoxins, especially where human disorders are concerned. An overview of bioassays in use in mycotoxin research is also given in this report. Most bioassays, however, are not suitable for investigating specific health effects. For this reason, more specific assays ought to be designed.

Contents

Samenvatting

Blootstelling aan (toxische metabolieten van) schimmels vormt een bedreiging voor de gezondheid van mens en dier. Met het uitvoeren van een volledige risico analyse kan een inschatting gemaakt worden van het werkelijke risico op negatieve gezondheids-effecten. In een eerder verschenen rapport[1] worden in het kader van een gevaren analyse de schimmel genera *Aspergillus*, *Penicillium*, *Fusarium* en *Alternaria* genoemd als de meest belangrijke genera in verband met besmetting van granen en graanproducten.

Dit rapport bevat, als onderdeel van een karakterisering van het gevaar, een overzicht van negatieve gezondheids-effecten bij mens en dier veroorzaakt door mycotoxinen afkomstig van schimmels van eerder genoemde genera. Aangezien geen kwantitatieve toxicologische gegevens zijn opgenomen kan niet worden gesproken van een volledige karakterisering van het gevaar.
Mycotoxinen werden in dit rapport opgenomen als:

1) ze in het eerdere rapport[1] aangaande het vóórkomen en de detectie van mycotoxinen in granen en graanproducten genoemd worden; en
2) uit de literatuur van de afgelopen 10 jaar duidelijk naar voren komt dat onderzoek wezenlijk nieuwe inzichten omtrent het vóórkomen en de werking heeft gegenereerd (vnl. door *Fusarium* en *Alternaria* species geproduceerde toxinen); of
3) de mycotoxinen al dusdanig lang onderkend worden als zijnde schadelijk voor de gezondheid dat niet-bespreken leidt tot onvolledigheid (vnl. door *Aspergillus* en *Penicillium* species geproduceerde toxinen).

De *Fusarium* toxinen fumonisinen en trichothecenen worden heden ten dage uitgebreid bestudeerd. Door de grote hoeveelheid experimentele gegevens is de aandacht voor deze twee groepen toxinen in dit rapport relatief groot.

De aard van de mogelijke negatieve gezondheids-effecten is zeer uiteenlopend: groeistoornissen, hepatoto- en nephrotoxiciteit, immunomodulatie, carcinogeniteit en mutageniteit vormen slecht een gedeelte van de aandoeningen die door mycotoxinen veroorzaakt kunnen worden. Het grootste gedeelte van de aandoeningen die door mycotoxinen bij dieren veroorzaakt worden is min of meer uitgebreid bestudeerd in dier-experimenten. Bij de mens worden slechts een klein aantal

aandoeningen aan mycotoxinen toegeschreven, hoewel soms het bewijs daarvoor niet onomstotelijk geleverd is. Zodoende vergt het achterhalen van de invloed van mycotoxinen op humane gezondheid nog veel onderzoek. Onderzoek, middels biologische testsystemen (bioassays), specifiek gericht op effecten die bij de mens worden waargenomen en het vaststellen van dosis-respons relaties zouden kunnen bijdragen aan het vaststellen van de relatieve bijdrage aan de waargenomen ziekten (afwijkingen) bij de mens.

In dit rapport wordt tevens een overzicht gegeven van de voorhanden zijnde bioassays. De meeste van de besproken assays zijn echter bedoeld om kwalitatief schadelijkheid[a] van een chemische verbinding vast te stellen en niet om specifieke effecten als carcinogeniteit of immunotoxiciteit vast te stellen. Daartoe zullen andere test systemen moeten worden ontwikkeld.

[a] Onder schadelijkheid worden ook effecten als mutageniteit en carcinogeniteit verstaan.

Summary

Exposure to moulds and their (toxic) metabolites (mycotoxins) is a threat for human and animal health. A risk analysis sheds light on the actual risk of adverse health effects. In a previously published report[1], being a hazard identification, the genera *Aspergillus*, *Penicillium*, *Fusarium* and *Alternaria* were designated the most important genera with respect to contamination of cereals and cereal products.

In this report, as part of a hazard characterization, an overview is given on adverse health effects in man and animals caused by moulds and mycotoxins of the above mentioned genera. As no quantitative toxicological data are presented this report can not be considered as being an entire hazard characterisation.

The selection of mycotoxins discussed in this report is based on:

1. data from an afore mentioned report[1] listing occurrence of toxins in cereals and cereal products; and
2. the literature of the last ten years when clear that essential new views on the occurrence and action have been gained (mainly toxins produced by *Fusarium* and *Alternaria* species); or
3. the mycotoxins having a reputation for posing health problems that not-mentioning leads to incompleteness (mainly toxins produced by *Aspergillus* and *Penicillium* species).

Based on the most recent literature it is obvious that *Fusarium* toxins, and especially fumonisins and trichothecenes, are being studied extensively at the moment. Due to the amount of experimental data, much attention is paid to toxins produced by this genus.

The nature of adverse health effects is very diverse: growth disorders, immunomodulation, carcinogenicity, and mutagenicity are just some of the disorders that can be caused by mycotoxins. Most of the disorders occurring in animals that can be conclusively attributed to moulds and mycotoxins have been studied more or less extensive in experimental animals. As only a few disorders in man can be conclusively attributed to mycotoxins, much research is needed to further investigate the role of mycotoxins in human health. Research, using bioassays, directed to health effects seen in humans and determination of dose-response relations can contribute to assess the contribution of mycotoxins to health effects in humans. An overview of bioassays in use in mycotoxin

research is also given in this report. Most bioassays, however, are meant for general toxicity rating and are not suitable to investigate specific health effects. Therefore, more specific assays ought to be designed.

1. Introduction

Fungi are ubiquitous microorganisms, which can be classified into three groups dependent on their properties and activities:

1. Useful: like for example producers of antibiotics
2. Harmful: as causing agents of infections, allergies and mycotoxicoses.
3. Non-harmful.

Mycotoxicoses comprise toxic, teratogenic, carcinogenic and immunomodulating effects caused by a great number of (combinations of) secondary metabolites produced by fungi [2]. The main source of contact for humans and animals is the consumption of contaminated food and feed.
Mycotoxins not only cause severe pathological aberrations to organs due to chronic or prolonged exposure [3-5], but also acute effects like vomiting through food intoxication [6].

Risk assessment, as described by the Codex Alimentarius Commission [7] can be employed to give an answer to the importance of (metabolites of) fungi with respect to human and animal exposure to fungi and fungal products.

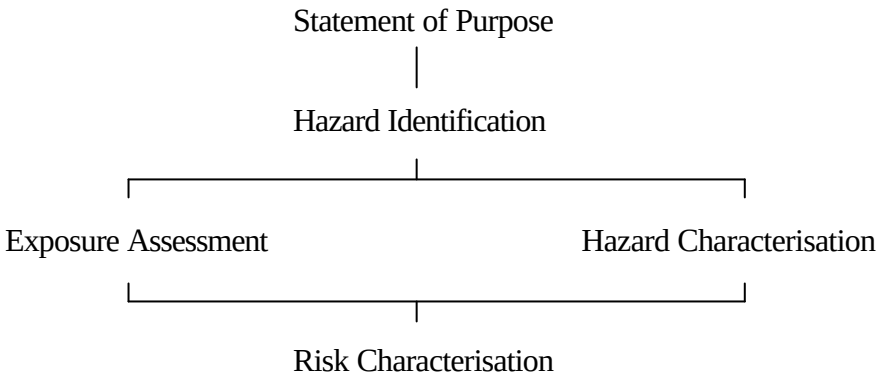

Figure 1: Scheme describing the separate steps for risk assessment.

For this project the Statement of Purpose has been described as a risk assessment study to the exposure to fungi and (toxic) fungal metabolites occurring in cereals and cereal products and the implications for public health.

In a previous report[1] an inventory has been made of the fungi and metabolites that can be present in or on cereals and that could pose a problem for public health (Hazard Identification).

This report deals with Hazard Characterisation, an inventory of the (adverse) health effects in man and animals caused by (toxic) secondary fungal metabolites that can occur in or on cereals and grain products and with bioassays in use or to be used to investigate adverse health effects. As no quantitative toxicological data like effect levels are presented this report should be seen as part of a hazard characterisation. The inventory is based on literature data from 1989 until 1998; inclusion of earlier literature data is based on quotes in the literature of the indicated period.

1.1 Approach / Design of this report

The most important mould genera with respect to contamination of cereals and cereal products, are *Aspergillus*, *Penicillium*, *Fusarium* and *Alternaria* [1]. Species of the genera *Aspergillus*, *Penicillium* and *Alternaria* are related mostly to storage. Contamination of cereals with species of the genus *Fusarium* is considered to occur during growth of host plants in the field. Some of the toxins produced by these four genera will be discussed in more detail in this report.

Fusarium toxins, and fumonisins and trichothecenes in particular, have been identified in recent years to cause adverse health effects and have therefore become important topics for research. That is the main reason why so much attention is paid to these toxins in this report in relation to the toxins produced by *Aspergillus*/*Penicillium*. Because of the resemblance of the most important *Alternaria* toxin AAL (*Alternaria alternata f.sp. lycopersici* toxin) to fumonisins (*Fusarium toxins*) with respect to toxicity and structure, this toxin will not be discussed separately but in conjunction with fumonisins.

The structure of each chapter is more or less the same: a complete list of items that may be discussed can be found at the end of this introductive chapter. The items actually being discussed per (group of) toxin(s) depend on the available information.

A separate chapter deals with general and effect-specific bioassays in use or to be used in conjunction with research to the health effects of mycotoxins.

The amount of literature concerning mycotoxins in general, natural or experimentally induced (adverse) health effects caused by mycotoxins and bioasays concerning mycotoxins is very extensive. Hence this report is no exhaustive compilation on the subject.

List of items discussed in the various chapters:

- Introduction
- Association with animal disease
- Association with human disease
- Carcinogenicity (animal)
- Carcinogenicity (human)
- Teratogenicity (animal)
- Teratogenicity (human)
- Mutagenicity
- Cytotoxicity (animal)
- Cytotoxicity (human)
- Immunotoxicity (animal)
- Immunotoxicity (human)
- Genotoxicity (animal)
- Genotoxicity (human)
- Neurotoxicity (animal)
- Nephrotoxicity (animal)
- Haematotoxicity (animal)
- Haemototoxicity (human)
- Pulmonary disorders / Respiratory disorders
- Growth disorders (animal)
- Reproductive disorders (animal)
- Cardiovascular effects (animal)
- Allergenicity (animal)
- Bioassays
- Conclusive remarks

2. Trichothecenes

Trichothecenes are a group of secondary fungal metabolites mainly produced by *Fusarium* species. Table 1 gives an overview of trichothecenes produced by various *Fusarium* species, as far as known in 1983. Meanwhile numerous other *Fusarium* species have been found to produce certain trichothecenes.

Table 1. Relation between Fusarium species and trichothecenes (copied from [8]).

Fusarium species	Trichothecenes
F. graminearum (*Gibberella zeae*)	deoxynivalenol, 3-acetyldeoxynivalenol, nivalenol, fusarenon-X
F. culmorum	deoxynivalenol, 3-acetyldeoxynivalenol
F. equiseti	diacetoxyscirpenol, neosolaniol, nivalenol, fusarenon-X
F. semitectum	diacetylnivalenol
F. acuminatum	T-2 toxin, HT-2 toxin, diacetoxyscirpenol, neosolaniol
F. nivale	nivalenol, fusarenon-X, diacetylnivalenol
F. sporotrichioides	T-2 toxin, HT-2 toxin, diacetoxyscirpenol, neosolaniol
F. sulphureum	T-2 toxin, diacetoxysirpenol, 3-acetyldiacetoxyscirpenol
F. poae	diacetoxyscirpenol, neosolaniol
F. oxysporum f. sp. *carthani*	T-2 toxin
F. solani var. *coerulum*	diacetoxyscirpenol
F. moniliforme	T-2 toxin, diacetoxyscirpenol

The basic structure of trichothecenes is shown in figure 2.

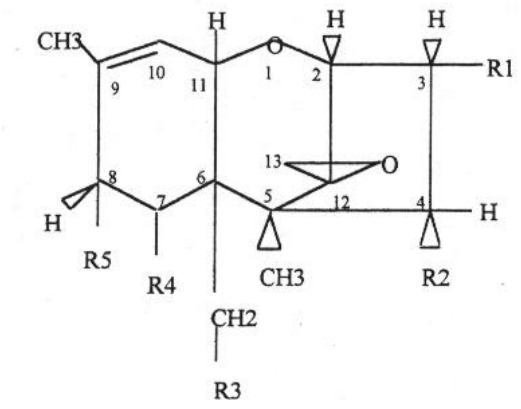

Figure 2. Basic structure of trichothecenes.[9]

Several structural features affect the biological activity of trichothecenes. The presence of an unsaturated bond at the C9-C10 position, integrity of the 12,13 epoxide ring, substitution of hydroxyl or other groups at appropriate positions on the trichothecene nucleus, and the structure and position of a side chain can all influence the extent of cytotoxicity and protein synthesis inhibition[10].

The trichothecenes can be divided into two subgroups: the type A trichothecenes are the compounds that have an isovaleryl, hydrogen or hydroxyl at the C8 position (e.g. T-2 toxin), and the type B trichothecenes that have a carbonyl at the C8 position (e.g. deoxynivalenol) [10].

Although more than 150 different trichothecenes, all deriving from the same basic molecule, trichodiene [11 12], have been identified, the most important with respect to human and animal health are T-2 toxin, deoxynivalenol (DON, vomitoxin), diacetoxyscirpenol and to a lesser extent HT-2 toxin and fusarenon-X [13]. These will be looked at closer.

Other fungi than *Fusarium* capable of producing trichothecenes

Stachybotrys (S.) atra, a saprophytic and worldwide-distributed fungus, is known to be able to affect horses, calves, swine and sheep and produces macrocyclic trichothecenes. Although to date some 11 *Stachybotrys* species have been identified, *S. atra* is the only species reported to produce trichothecene toxins.

Other fungi able to produce trichothecene toxins are *Myrthecium (M.) verrucaria* and *M. roridum*, *Trichothecium* species, *Trichoderma* species and *Cephalosporium crotocinogenum*. The significance of their metabolites in human and animal health, however, has not yet been clarified [8].

2.1 Fusarenon-X

Introduction

Fusarenon-X (FUS), a type B trichothecene, was first isolated in 1968, and characterized in 1969 [14]. It is not mentioned very often in the literature, whereas the main interest for trichothecenes is with deoxynivalenol, T-2 toxin and HT-2 toxin.

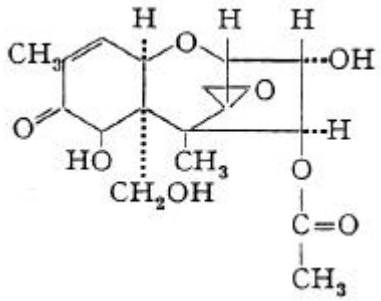

Figure 3. Structure of Fusarenon-X [14].

Carcinogenicity (animal)

The International Agency for Research on Cancer (IARC) concluded that insufficient experimental data were available for determining the carcinogenicity for laboratory animals due to inadequate studies [15].

Carcinogenicity (human)

No epidemiological data were available to assess the carcinogenicity of FUS for humans [15].

Teratogenicity (human)

Although there is a risk of exposure to FUS by consumption of contaminated food no data are available to evaluate the teratogenicity or chromosomal effects of FUS in humans [15] .

Cytotoxicity (animal)

When administered intraperitoneally (1.5 mg/kg) to 6-week-old Wistar rats mitotic inhibition was seen 2 hours post administration (PA). The peak toxic effect was seen 3 hours PA with marked

apoptosis from gastric epithelium cells. These findings were confirmed by in situ detection of DNA breaks which also reached a peak 3 hours PA [16].

FUS is not just toxic to gastric epithelial cells, but also to murine thymocytes. When administered intraperitoneally three times FUS caused severe thymic atrophy, with the thymic cortex almost completely disappearing. Primary targets were the $CD4^+CD8^+$ thymocytes. A single injection of FUS caused DNA shearing in murine thymocytes [17].

Immunotoxicity (animal)

Like other trichothecenes FUS inhibits lymphocyte blastogenesis. This effect was determined by measuring the incorporation of [^{3}H]-thymidine. The mean ID_{50}[a] from experiments, in which unstimulated, ConA[b] and LPS[c] stimulated cells were used, was 38 ng/ml [18].

Bioassays

The toxic action of FUS can be established with the yeast bioassay. When applied on a small disc (6.35 mm in diameter) and placed on a culture plate containing *Kluyveromyces marxianus* the detection limit for FUS is 10 µg [19].

FUS has anti-viral activity, through affecting replication of Herpes Simplex Virus Type 1 and 2 (HSV-1 and 2, respectively) rather than inhibiting adsorption of the virus particles. The EC_{50}[d] for FUS was determined to be 56 ng/ml for HSV-1 and 26 ng/ml for HSV-2 [20].

Isshiki et al. [21] compared several tests to assess the cytotoxicity of a number of *Fusarium* toxins. For these tests human hepatoblastoma cells (HuH-6KK) were used. They concluded that a chemiluminescence test was the easiest and fastest to perform. With this test the IC_{50}[d] for FUS was set at 0.3 µg/ml.

Conclusive remarks

Fusarenon-X, a trichothecene toxin produced by *Fusarium* species, is toxic to murine thymocytes, lymphocytes, and gastric epithelial cells and to human hepatoblastoma cells. All these findings come from studies with cell lines. In correlation with the effect on gastric epithelial cells the acute toxic

[a] ID_{50} = the inhibitory dose that causes 50% reduction of [^{3}H]-thymidine incorporation
[b] ConA = Concanavalin A, a T-lymphocyte mitogen
[c] LPS = lipopolysaccharide, a B-lymphocyte mitogen
[d] EC_{50} /IC_{50} = the 50% effective/inhibitory concentration

effect to cats and ducks, such is vomiting [22], may be considered. The effects to thymocytes and lymphocytes can be categorised as immunotoxic. As also effects have been found in human hepatoblastoma cell lines, it is interesting to resolve the effect of Fusarenon-X on human thymocytes and lymphocytes. Extrapolation to possible immunotoxic effects in vivo would thus be easier.

2.2 Deoxynivalenol

Introduction

Deoxynivalenol (DON, vomitoxin), the structure of which is shown in figure 4, is a trichothecene toxin produced by *Fusarium graminearum* amongst other species.

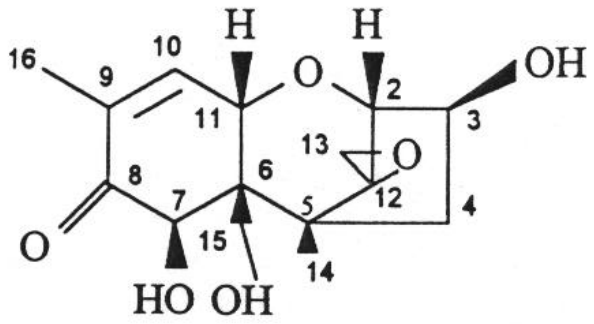

Figure 4. Structure of deoxynivalenol [10].

DON is prevalent worldwide in crops used for food and feed production. Although DON is one of the least acutely toxic trichothecenes, it should be treated as an important food safety issue because it is a very common contaminant of grain. The main overt effects at low dietary concentrations appear to be decreased growth and a reduction in food consumption (anorexia), while higher doses induce vomiting (emesis), immunotoxic effects and alterations in brain neurochemicals [10].

Association with human disease

Outbreaks of toxicosis associated with consumption of mould-contaminated wheat and corn and related to the presence of *F. graminearum* have been reported in Japan (red mould disease), India (deoxynivalenol toxicosis, 0.35 to 8.38 ppm DON), and China (fusariotoxicosis, 0.34 to 92.8 ppm DON)[23]. Evidence for carcinogenicity of toxins derived from *F. graminearum* in humans or for DON on experimental animals is inadequate, based on the 1993 World Health Organisation evaluation [10].

Teratogenicity (animal)

Although in white leghorn hens no significant changes in weight gain, food intake, egg production, fertility, hatchability were discovered when fed 120 to 4900 µg DON/kg feed, the number of developmental anomalies seen increased with the concentration of DON with a peak at 3100 µg DON/kg feed. Anomalies consisted of minor malformations like delayed ossification and major malformations like cardiac anomalies [24].

Cytotoxicity (animal)

At a concentration of 100 µg/ml DON was strongly cytotoxic to rat hepatocytes, that were stimulated to proliferate through the addition of growth factor. In chromosomal aberration assays pronounced dose dependent effects were observed with DON [25].

Immunotoxicity (animal)

Growing pigs were fed diets containing DON-contaminated oats at 0.6, 1.8 and 4.7 mg/kg feed. The development of primary and secondary antibody titers after injections of five different antigens (human serum albumin, sheep red blood cells, paratuberculosis vaccine, tetanus toxoid, and diphtheria toxoid) was recorded. A significant dose-dependent lowering of secondary antibody response to tetanus toxoid was observed. No significant differences in titres to the other antigens were observed. Moreover DON appears to increase the influence of mitogens on lymphocytes. A significant higher response to one of the three mitogens used (phytohaemagglutinin, PHA) was observed in the high DON group compared to the low DON group [26].

In another study using young pigs exposed orally to DON at levels of 3 mg DON/kg diet a delayed response in peak titers to sheep red blood cells was observed [27].

Interleukin (IL)-2 is an inducer of IL-5 and IL-6 that are key cytokines in IgA synthesis. DON super-induces IL-2 gene expression in murine EL-4 thymoma cells in a dose dependent manner at levels of 50 to 250 ng/ml. In accordance with the elevated mRNA levels, IL-2 production was significantly elevated [28]. Ouyang et al. [29] investigated the mechanism of IL-2 superinduction with an emphasis on transcription factor NF-?B/Rel and found increased binding activity with increasing DON concentrations.

Not only IL-2 mRNA expression is induced by the oral exposure to DON, also mRNA expression of other cytokines is (non-) significantly induced [30 31]. These results indicate that elevated cytokine

expression may play a contributory role in the pathophysiologic and immunologic effects of DON. Similar results were also found in an in vitro system utilising a murine macrophage cell line [32].

Macrophage cells, obtained from 21-day-old turkeys, were dose dependently affected by DON. 50 µg/ml DON caused a markedly decreased adherence to the glass surface used to grow the macrophages. Also phagocytosis of opsonized SRBC (sheep red blood cells) was decreased by this concentration of DON. When using a concentration of 200 µg DON/ml also the phagocytosis of unopsonized SRBC was decreased. Another effect was the increasing percentage of damaged macrophage cells with increasing DON concentrations [33]. Ji and coworkers investigated how DON affects the macrophage regulatory and effector function in order to improve understanding of mechanisms by which DON affect the immune system. A murine cell line (RAW 264.7) was used to assess the effects of DON on proliferation and the production of nitric oxide (NO), hydrogen peroxide (H_2O_2) and cytokines. Without stimulation by lipopolysaccharide (LPS) or interferon (IFN)-? DON (25 – 50 ng/ml) markedly decreased proliferation. No effects however were recorded on the production of NO, H_2O_2 and cytokines under these circumstances. Stimulation by LPS or IFN- ? was needed to affect the production of NO, H_2O_2 and cytokines [tumour necrosis factor (TNF)-a and interleukin (IL)-6]. DON appears to be capable of selectively and concurrently up or downregulate critical functions with activated macrophages [34].

Studies in mice, fed diets containing 2, 10, 25 or 50 ppm DON, revealed that mice fed at 25 ppm DON had more than 17-fold increased IgA levels after 24 weeks of exposure compared to control mice. Also serum IgA exhibited a marked shift from primarily monomeric IgA to primarily polymeric IgA during DON treatment. Splenocytes from mice exposed to DON showed significantly increased IgA production with and without mitogen stimulation. An accumulation of glomerular IgA was observed using immunofluorescence staining. The reactions in mice caused by DON, dysregulation of IgA production and accumulation of glomerular IgA, resemble the characteristics of human IgA nephropathy [35].

Neurotoxicity (animal)

Neurological studies are mainly carried out to further investigate the anorectic mechanism of action of DON. Intravenous (iv) administration of DON (2.5 mg/kg) to pigs resulted in significantly different levels of norepinephrine, dopamine and 5-hydroxytryptamine in hypothalamus, frontal

cortex and cerebellum compared to controls. The alterations found are, however, not indicative of known neurochemical changes associated with chemical-induced anorexia [36].

In another study pigs were given DON either intravenously (6 times 10 µg/kg) or intragastrically (6 times 30 µg/kg). Catecholamine levels were monitored. After intragastrical administration a rapid increase occurred, and an elevated level was seen for up to 20 hours post-dosing. Levels were also elevated after intravenous dosing, although to a lesser extent and lasting up to 6 hours post-dosing. The elevated levels are indicative of increased serotoninergic activity, and indicative to the theory that links elevated brain serotonin turnover to a decreased feed intake [37]. DON, however, was found to be ineffective in blocking membrane binding sites for 5-hydroxytryptamine (5HT, serotonin), an indole neurotransmitter believed to be involved in DON-regulated effects [38].

Haematotoxicity (animal)

Barrows fed diets contaminated with DON (4.5 mg/kg feed for 28 days) showed no signs of altered haematological values [39]. In contrast to what is known about trichothecenes as being strongly toxic to human CFU-GM cells DON does not exhibit any effect on the proliferation of human BFU-E cultures (red blood cells progenitors) [9].

Growth disorders (animal)

Deoxynivalenol fed to broiler chicks from day one to day 21 after hatching caused a slight reduction of body weight (BW) gain, namely 2 %, when diets containing 15 mg/kg were used. When fed a diet containing both DON and fumonisin B_1 reduction of BW gain increased to 19%, indicating that DON only contributes slightly to the reduction in BW gain. Especially as the researchers had no indication of additive toxicity or even synergism [40].

Another study using broiler chicks from day one to day 21 after hatching indicated that DON at 16 mg/kg feed caused hardly any reduction in BW gain nor was feed consumption impaired dramatically [41].

In a 21 day experiment feeding immature swine blends of mycotoxin contaminated cereals containing both DON (0 to 2 mg/kg) and fusaric acid (3 to 16 mg/kg) a reduction in feed intake and reduced BW gain were observed. Both DON and fusaric acid appeared to be responsible for the reduction in BW gain and feed intake [42].

When growing pigs were fed diets containing DON-contaminated oats at 0.6, 1.8 and 4.7 mg/kg feed the pigs receiving the medium and high dose showed visible reduced appetite. Also the average weight gain in the first 8 weeks of the experiment was reduced in the medium and high dosage groups and the time needed to reach the slaughterweight of 100 kg was prolonged in the medium and high dosage groups [26].

Feeding of DON-contaminated diets (4.5 mg/kg feed for 28 days) did not affect the final BW and BW gain of barrows. But a diet containing both DON and fumonisin B1 (3.7 and 56 mg/kg feed respectively) decreased final BW and BW gain of barrows drastically [39].

During a 6-week feeding trial, effects of low dietary DON (0, 0.1, 1, and 10 ppm) on feed consumption and BW gain were investigated in male mice. The authors found significant weight gain reduction and significant reduced transfer of glucose in isolated perfused jejunum segments in the 10 ppm group. Also in the 10 ppm group the manganese and molybdenum content in liver tissue was reduced compared to the other groups. These findings led to the conclusion that subchronic ingestion of DON, in concentrations occurring in contaminated food and feed resulted in weight gain reduction and impairment of intestinal uptake and transfer [43].

Also horses have been subjected to feeding trails to investigate whether they are prone to negative effects caused by DON contaminated barley. During and after a 40-day period, receiving 1,27 kg DON-contaminated barley (36-44 ppm) every 12 hours no adverse effects were registered. Also no changes in serum IgA concentration, which is a sensitive indicator of DON effect, were measured [44].

Bioassays

Like Fusarenon-X DON displays anti-viral activity. When added to cultures of Hep-2 cells, DON effectively inhibited the replication of Herpes Simplex Virus type 1 and type 2 (HSV-1 and HSV-2). The EC_{50}[a] for DON was 160 ng/ml for HSV-1 and 94 ng/ml for HSV-2 [21].

A series of experiments was conducted to determine the feasibility of using mice to screen for possible dietary mycotoxin interactions before testing them with swine. Although it was established that effects on BW caused by DON were more pronounced in a dose-related manner when exposure to contaminated diets started at day 21 of age rather than at day 28, in both age groups

[a] = EC_{50} = the 50% effective concentration

weight gain response in relation to exposure of DON was linear, similar to that seen in swine. The authors therefore concluded that compared to swine, mice display similar dose-dependent linear responses in feed consumption and weight gain due to the presence of dietary DON and that therefore the mouse bioassay is a suitable model for short term experiments [45]. In an investigation of later date the same investigators found that male mice are more sensitive to dietary DON than female mice. Also the feed intake of a low energy diet was higher than that of a high-energy diet, which leads to a higher consumption of DON [46].

DON can directly influence cells of the immune system. Reduction of lymphocyte response in animals exposed to DON was proven. Measurement of the activity of these immune cells is possible in an in vitro test. Proliferation of the cells is measured by determination of the new synthesised DNA in multiplied cells where the thymidine analogue BrdU is incorporated. BrdU can be detected enzymatically in a colorometric assay [47].

Conclusive remarks

Deoxynivalenol (DON), a trichothecene toxin produced predominantly by *Fusarium graminearum*, is known to cause both acute and chronic adverse health effects in man and animals. Several chronic effects in animals have been found such as growth inhibition in chickens and pigs, immunotoxicity in pigs and mice, and damage to genetic material in rat cell lines. Also, associated with growth inhibition, neurotoxic effects have been found in pigs.

In man acute toxicity has been found, causing vomiting and irritation to the respiratory tract. Most prominent was an outbreak in India in the late 80's caused by wheat contaminated with a DON-producing *Fusarium* strain.

Although most toxic effects have been measured in animals, DON can also cause adverse health effects in man. This statement is underlined by an Acceptable Daily Intake (ADI) level of 1 µg/kg in the United States, although Ehling [48] showed that the International Estimated Daily Intake for DON exceeds this ADI by 3,5 to 89 fold.

To further unravel the toxicity of DON to man more research is necessary, facilitation of which can be accomplished by using human cell lines.

2.3 T-2 Toxin

Introduction

T-2 toxin, of which the structure is shown in figure 5, is regarded as the most toxic member of the trichothecene family.

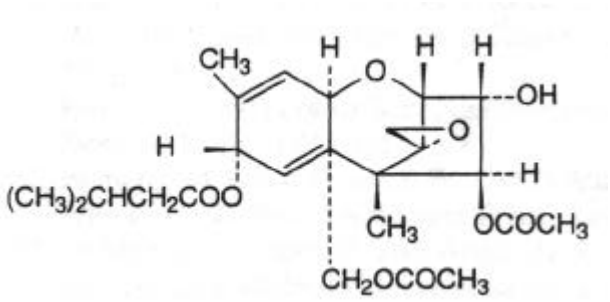

Figure 5. Structure of T-2 toxin [49].

T-2 toxin is the highest toxic compound of all trichothecene toxins [50]. Its presence has among others been demonstrated in maize and wheat. Like other trichothecene toxins the mode of action of T-2 toxin is most often characterised by gastrointestinal problems and feeding problems. Moreover, T-2 toxin has been associated with feathering problems in chickens and decreased egg production.

Association with human disease

Alimentary toxic aleukia (ATA) is a disease that strikes man with the following possible clinical features: leukopenia, agranulocytosis, bleeding from the nose, throat and gums, necrotic angina, haemorrhagic rash, sepsis, exhaustion of the bone marrow and fever. The first reported outbreak of the disease was in Russia in 1913 (Orenburg district) and has been associated with the consumption of *Fusarium* contaminated grain.

Over the years several investigators proposed several different *Fusarium* metabolites as being the cause of the disease. In experiments conducted by Lutsky and Mor (cited in [23]) orally administered T-2 toxin produced effects similar to the disease syndrome of ATA in man [23].

Another disease that has been associated with T-2 toxin is pellagra, a deficiency disease due to insufficient intake or failure of the body to absorb the complex vitamin niacin or its amide. The

evidence for involvement of mycotoxins as primary causal agent is, however, entirely circumstantial [23].

Carcinogenicity (animal)

To evaluate the hepato-carcinogenic properties of T-2 toxin rats were given doses of 2 and 5 ppm T-2 toxin, some without pre-treatment and some after being injected with diethylnitrosamine. In rats from both groups no evidence was found for direct carcinogenicity of T-2 toxin, nor for a carcinoma enhancing effect by T-2 toxin [49].

Immunotoxicity (animal)

To assess immunotoxic effects of several mycotoxins the murine thymoma cell line EL-4 was used. Cells were stimulated in the presence of mycotoxins in various concentrations and supernatants were analysed for interleukin (IL)-2 and IL-5. T-2 toxin at a concentration of 5 ng/ml or more totally suppressed the IL-2 and IL-5 production. Concurrently a marked depression of A570 was measured in the MTT-assay, indicating very poor proliferation and cell viability [51].

Mice, exposed to T-2 toxin for 5 consecutive days, showed thymic atrophy on the second day following cessation of dosing characterised by significant decrease in total number of cells with phenotypes defined by CD4 and CD8 cell surface antigen expression. Further, the distribution of thymocytes within these phenotypes was significantly altered. Increased percentages of $CD4^-8^-$ and decreased percentages of $CD4^+8^+$ cells in thymuses from treated animals suggested that T-2 toxin may inhibit thymocyte maturation [52]. Also mice treated with a single injection of T-2 toxin showed time and dose dependent atrophy with a marked decrease of the $CD4^+CD8^+$ thymocyte population. Maximal atrophy was induced by a dose of 1.75 mg T-2 toxin/kg intraperitoneally injected by day 3 with complete recovery by day 7 [53].

Subchronic T-2 toxin treatment of timed-pregnant B6C3F1 mice resulted in significant and selective depletion of foetal liver cells expressing low levels of surface CD44 and CD45 antigens, suggestive of possible lymphoid progenitor cell sensitivity to this agent [54].

Neurotoxicity (animal)

T-2 toxin has been shown to affect the central nervous system. There exists, however, a lack of consensus on the central effects, which is in part caused by the plethora of experimental designs and

analytical techniques employed. Therefore Wang et al. [55] designed a study to characterise in more neurochemical detail the dose-related effects of T-2 toxicity on regional metabolism of selected brain monoamines and their metabolites. They dosed male rats orally with T-2 toxin at 0.1, 1.0 and 2.5 mg/kg BW. Analyses were carried out 2, 6 and 10 hours post-dosing. T-2 toxin treatment increased serotonin levels throughout the brain, and produced a transient local increase and decrease at other sites of norepinephrine. No regional changes of epinephrine or dopamine were observed. With a dose of 0.1 mg/kg BW, T-2 significantly affected brain monoamines. Few other treatment differences were observed.

Haematotoxicity (animal)

Trichothecenes are known to provoke syndromes that resemble ATA[a] closely. However, administration of purified trichothecenes to experimental animals failed to reproduce the ATA symptomatology. Prolonged dietary exposure of mice to T-2 toxin led initially to extensive haematological damage, followed by restoration of erythropoietic activity several weeks later, under continuous exposure to the toxin. Further investigation of the influence of a single dose of T-2 toxin on the erythropoietic system of mice was carried out by measuring the uptake of radioisotopic iron into bone marrow and spleen as a function of time. After a potent inhibition of iron uptake there was a remarkable recovery of the system in the spleen after 48-72 hours. Recovery in the marrow took significantly longer, namely 21 days. After splenectomy bone marrow erythropoietic activity was repaired rapidly, indicating that under the described experimental conditions no irreversible damage was done to the marrow haematopoietic cells [56]. The inhibition of radioisotopic ^{59}Fe incorporation by erythrocytes treated with T-2 toxin was also studied by Faifer et al. [57]. They state that this method can be a sensitive means for studying the risk of erythropoietic injury produced by dietary exposure to T-2 toxin specifically and trichothecene mycotoxins in general.

T-2 toxin also affects the phospholipid turnover in bovine platelets. In non-stimulated platelets T-2 toxin exposure leads to a marked increase of phosphatidic acid, parent compound of the structurally and metabolically important phosphatides. Treatment of platelets with platelet activating factor leads to metabolism of phosphatidic acid into phosphatidyl inositol. This process, however, is inhibited by exposure to T-2 toxin, indicating that T-2 toxin apparently impedes the action of a specific phospholipase [58].

Haematotoxicity (human)

In order to investigate the possibility of using rat CFU-GM[b] cells as a model for assessment of T-2 toxin toxic effects and the mode of action on haematological progenitors Lautraite et al. [59] compared the sensitivity of rat and human cells to T-2 toxin. Although IC_{50}[c] values did not differ significantly, there is a marked difference in the expression of the cytotoxicity. A sharply appearing cytotoxic effect was observed on rat progenitors, while a partial cytostatic effect was observed in human cells, thus making the rat model not the right model for predicting T-2 toxin toxic effects on human haematopoietic progenitor cultures. The effect of T-2 toxin on red blood cellprecursor proliferation and differentiation was measured by incubating human erythroblastic progenitors (BFU-E)[d] in the presence of T-2 toxin. The toxin caused decreased cell growth, different pigmentation (which means an effect on haemoglobin synthesis), and decrease of the porphyrin content at one T-2 toxin concentration. Porphyrin is a precursor of heme, necessary for the binding if iron by haemoglobin [9].

Pulmonary disorders / Respiratory disorders

Respiratory tract cilia and their movement represent one of the most important biological barriers between human organisms and the environment, responsible for clearance of physical, chemical or biological harmful substances from the respiratory tract. In trachea from one-day-old chicks T-2 toxin stopped ciliary movement at a concentration of 20 – 0.6 mg/l after 2 days and at 300 – 30 µg/l after 3 days of incubation, thus affecting one of the most important barriers between an organism and its environment [60].

Growth disorders (animal)

Male broiler chicks were fed diets containing 5 mg T-2 toxin/kg from day of hatch to 19 or 21 days of age. During this time BW gains were reduced 18% compared to control animals[40]. In another report using male broiler chicks a decreased BW gain is mentioned too after feeding 6 mg T-2 toxin/kg diet. Also oral lesions were present in the animals fed on the t-2 toxin diet [61].

Also female turkey poults showed reduced BW gain, 26% compared to controls, when fed a diet containing 5 mg T-2 toxin/kg from the day of hatch to 21 days of age. Moreover, T-2 toxin induced

[a] ATA = alimentary toxic aleukia (see “illnesses”)
[b] CFU-GM = Colony Forming Unit – Granulocyte and Macrophage
[c] IC_{50} = concentration that leads to 50% inhibition
[d] BFU-E = Burst Forming Unit - Erythroid

rather severe oral lesions in the birds [62]. Mallard ducklings, 6-weeks-old, showed oral lesions and reduction of BW gain after being fed 2 ppm[a] T-2 toxin [63]. Like broiler chicks, turkey poults and mallard ducklings, ringneck pheasants also show decreased BW gain and mouth lesions when fed a diet containing T-2 toxin [64].

Deoxynivalenol (DON) is known to affect growth performances of e.g. mice. The effect of reduced weight gain by young mice becomes more pronounced when T-2 toxin is added to a diet containing DON. A dose dependent decrease in weight gain and food consumption was observed after 7 days on a diet contaminated with DON and T-2 toxin, whereas reduction of weight gain and food intake were detectable after 21 days on a diet contaminated with DON only [45].

As shown bird species differ in (the severity of) their reactions to T-2 toxin. Another example is a study described by Ruff et al. [65] in which it was clear that weight gain reduction, increased food conversion ratio and severity of mouth lesions were more pronounced in the bobwhite quail given a diet containing T-2 toxin than in the Japanese quail. When laying hens received a T-2 toxin contaminated diet oral lesions were observed as well as significantly reduced egg production. No effects, however, were seen on the BW. In combination with diacetoxyscirpenol the effects caused by T-2 toxin were intensified [66]. Tobias et al. [67] also studied the effect of T-2 toxin on the egg production of laying hens and found that the higher the T-2 toxin content of the diet the lower the amount of eggs produced and the hatchability of the eggs.

Cardiovascular effects (animal)

To assess the influence of T-2 toxin on the cardiovascular system white rats were given T-2 toxin intragastrically, in 1 to 8 doses corresponding with total amounts of 0.2 to 4 mg/kg BW. About one third of the animals reacted intensely to the administration of T-2 toxin and died within a few days after the first or after one of the subsequent treatments. These animals developed hunched posture, soiled underbelly from diarrhoea, and bleeding from body orifices. Some were found in coma. Autopsy revealed multiple aberrances in stomach, intestines, lungs, heart and blood vessels. Congested blood vessels were particularly conspicuous in the brain. In the rats that survived 12 to 27.5 months the cardiovascular system was often affected ranging from partly organised thrombi in the left auricle and ventricle of the heart to coronary arteries almost occluded by fibrinoid swelling of

[a] ppm = parts per million

the collagen [68]. Although cardiovascular disorders are quite common, no epidemiological data are available on the contribution of T-2 toxin.

Bioassays

The brine shrimp bioassay has been used to assess the toxicity and concentration that causes 50% mortality (LC50) of *Fusarium* extracts in general and T-2 toxin specifically. The LC50 value for t-2 toxin was 0.069 ppm [69].

To assess the toxicity of T-2 toxin the chick embryo test is used too. Vesely et al. [70] found that the site of administration is crucial to the sensitivity of the test. The closer the site of administration to the target tissues of the embryo, the higher the sensitivity and reproducibility of the test system .

T-2 toxin can directly influence cells of the immune system. Reduction of lymphocyte response in animals exposed to T-2 toxin was proven. Measurement of the activity of these immune cells is possible in an in vitro test. Proliferation of the cells is measured by determination of the newly synthesised DNA in multiplied cells where the thymidine analogue BrdU is incorporated. BrdU can be detected enzymatically in a colorometric assay [71].

As mentioned before the inhibition of incorporation of radioisotopic iron (^{59}Fe) is suggested by Faifer et al. [57] as a model for studying the erythropoietic injury caused by dietary exposure to T-2 toxin specifically and trichothecenes in general.

Conclusive remarks

T-2 toxin, a trichothecene produced by *Fusarium* species, is believed to be the causal agent in alimentary toxic aleukia (ATA) and pellagra, both human diseases. However, to date the direct relationships between the diseases and the toxin have never been established. Reactions of experimentally infected rats were similar to the symptoms seen in the case of ATA in man; the relationship between pellagra and mycotoxins being based completely on circumstantial evidence.

Besides posing a risk factor for human health, T-2 toxin causes several disorders in animals with growth effects in e.g. chicks being the most prominent. Immunotoxic and haematotoxic effects in experimentally exposed animals or cell lines have also been reported.

2.4 HT-2 Toxin

Introduction

HT-2 toxin is a trichothecene produced by several *Fusarium* species, among which *F. sporotrichioides* [8].

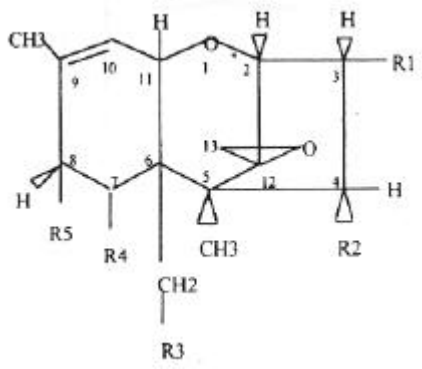

Figure 6. Structure of HT-2 toxin (R1=H, R2=H, R3=$OCOCH_3$, R4=$OCOCH_3$, R5=OH) [47].

Association with human disease

HT-2 toxin is one of the major metabolites of T-2 toxin and differs from T-2 toxin by one hydroxylgroup only. Ohta et al. [72] have described that the microsomal liver fraction has the ability of completely transforming T-2 toxin into HT-2 toxin within a few minutes. Accordingly, the toxic effect of T-2 toxin may be in part attributed to HT-2 toxin.

Cytotoxicity (animal)

The yeast *Kluyveromyces marxianus* has been used to assess the cytotoxicty of the trichothecene T-2 toxin. Also the combined action of T-2 toxin and HT-2 toxin has been evaluated using this yeast. When more or less equal amounts of both toxins are used the effect is either zero or at most antagonistic. When the amount of one toxin far exceeds the amount of the other and when the effect level is above 50% the interaction between the two toxins is considered synergistic [73]. This effect was stated by an outbreak of mycotoxicosis in 1984, described by Schlosberg (cited in [73]), in which there was a 94% reduction in egg production by a flock of laying hens. Concentrations of the toxins involved were 7.5 µM for T-2 toxin and 1.6 µM for HT-2 toxin.

Haematotoxicity (animal)

Phospholipids are constituents of eukaryote membranes. The parent molecule is phosphatidic acid (PA), which is metabolised to, amongst others, phosphatidyl inositol (PI). In non-stimulated bovine platelets this metabolic process is inhibited by HT-2 toxin, showed by a significant increase in PA concentration. The process of PA metabolism into PI after stimulation of the platelets with platelet activating factor is inhibited by exposure of the platelets to HT-2 toxin [58].

Haematotoxicity (human)

Human and rat haematopoietic progenitor cells are sensitive to T-2 toxin as shown by Lautraite et al. [59], with IC_{50}[a]-values not significantly different but with marked difference in the expression of cytotoxicity. Therefore they concluded that rat CFU-GM[b] cells were not to be used as model for human CFU-GM cells. In another study CFU-GM cells from both rats and humans were compared to determine whether HT-2 toxin exhibited the same toxicity as T-2 toxin. IC_{50}-values appeared to be almost identical [47].

The effect of HT-2 toxin on red blood cell proliferation and differentiation was measured by using human erythroblastic progenitors (BFU-E[c]) cultures. Growth percentages were decreased and varied between 71 and 79% compared to the control cultures. Neither the porphyrin[d] content nor the haemoglobin content were significantly influenced by HT-2 toxin [9].

Conclusive remarks

The adverse health effects caused by HT-2 toxin have to be considered in close relationship with effects caused by T-2 toxin, as HT-2 toxin is a derivative of T-2 toxin. The adverse health effects caused by HT-2 toxin are nearly similar to those caused by T-2 toxin. It is not unthinkable that some effects caused by T-2 toxin might also be found to be casued by HT-2 toxin if specific researches would be carried out.

Whether the effects, like haematoxicity, caused in animals also apply for man has to be further investigated .

[a] IC_{50} = concentration leading to 50% inhibition
[b] CFU-GM = Colony Forming Unit – Granulocyte and Macrophage
[c] BFU-E = Burst Forming Unit - Erythroid
[d] porphyrin = precursor of heme, necessary for the iron-binding capacity if haemoglobin

2.5 Diacetoxyscirpenol

Introduction

Diacetoxyscirpenol is a trichothecene toxin produced by several *Fusarium* species.

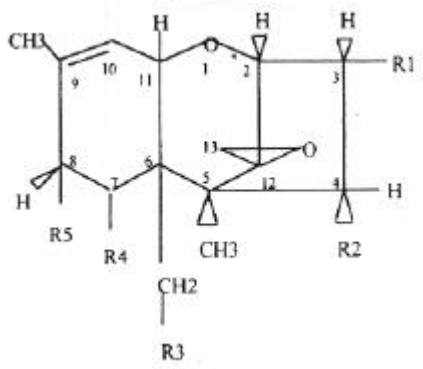

Figure 7. Structure of diacetoxyscirpenol (R1=OH, R2= OAc, R3=OAc, R4=H, R5=H)

Immunotoxicity (animal)

Diacetoxyscirpenol (DAS) has been described as having effect on the immune system [74]. In vitro studies have revealed that DAS suppresses the activity of murine peritoneal macrophages, with 2 ng/ml added to the medium reducing phagocytosis and 1 ng/ml added to the medium reducing microbicidal activity [75].

Haematotoxicity (animal)

Phospholipids are important constituents of eukaryote membranes. The parent molecule, phosphatidic acid, is metabolised amongst others into phosphatidyl inositol.

Trichothecenes are known to interfere with this metabolism. DAS is found to suppress the loss of phosphatidic acid and the appearance of phosphatidyl inositol after stimulation of bovine platelets by platelet activating factor [58].

Haematological effects have been described from a number of trichothecenes. Also diacetoxyscirpenol (DAS) causes haematological disorders. Rat granulo-monocytic progenitors were cultured in the presence of various concentrations of DAS (10^{-8}–$5x10^{-10}$ M). Most prominent was the total cytotoxicity of DAS at 10^{-8} M, while the IC_{50}[a] after 14 days is $6.2x10^{-9}$ M [76].

[a] IC_{50} = concentration at which growth of cells is reduced 50%

Haematotoxicity (human)

Human granulo-monocytic progenitors, obtained from umbilical cord blood, were exposed to various concentrations of diacetoxyscirpenol (DAS) varying from 10^{-7} to $5x10^{-10}$ M. Total cytotoxicity was achieved at 10-7 M, and IC_{50} was $7.6x10^{-9}$ M [77]. Also human erythroblastic progenitors were exposed to DAS at various concentrations. At a concentration of 10^{-7} M DAS only singular non-pigmented cells were seen, indicating almost total cytotoxicity. Lower concentrations partly inhibited cell growth, and decreased the amounts of haemoglobin and porphyrin [9].

Pulmonary disorders / Respiratory disorders

Cilia in the respiratory tract represent one of the most important biological barriers between human organisms and the environment. Impairment of the ciliary action can cause diseases. The influence of DAS on the ciliary movement was investigated by using tracheal rings from one-day-old chicks. DAS induced a ciliostatic effect after 2 days at a concentration of 30 µg/litre, which makes the compound one of the most effective trichothecenes with respect to disturbing ciliary movement in vitro [60].

Growth disorders (animal)

When turkey poults were fed a diet containing 4 mg diacetoxyscirpenol/kg feed from the day of hatch to 3 weeks of age, BW gain was reduced with 30%. The authors state that the amount of DAS used greatly exceeds the amount that is encountered under natural circumstances [78].

Growth effects after exposure to diacetoxyscirpenol have also been registered in laying hens. A dietary concentration of 2 mg DAS/kg resulted in oral lesions in about half of the hens used for the trial, and in a significantly reduced egg production and food intake [66].

Cardiovascular effects (animal)

Acute trichothecene intoxication can result in hypothermia and cardiopulmonary dysfunction. Most studies investigating the cardiovascular effect have been conducted in vivo, making them difficult to interpret. Kimbrough and Weekley [77] conducted studies on isolated aortic vascular rings to determine whether representative trichothecenes are capable of directly altering vascular smooth muscle tone. DAS did not cause relaxation of aorta precontracted by 30 mM potassium chloride

(KCl). Isoproterenol and sodium fluoride induce smooth muscle relaxation, which action is impaired in the presence of DAS.

Bioassays

Using the brine shrimp bioassay has assessed the toxicity of diacetoxyscirpenol (DAS). The LC_{50}[a] has been determined at 0.250 ppm [69].

Conclusive remarks

Diacetoxyscirpenol is not the most prominent representative of the trichothecenes. Yet, as the toxin is often produced in conjunction with other trichothecenes it is of importance. Also because there are indications that DAS alone causes effects like growth disorders and immunotoxicity, further elucidation of the adverse health effects to animal and man that can be caused by DAS remains important.

[a] LC_{50} = concentration that causes 50% lethality

3. Zearalenone

Introduction

Zearalenone (ZEA) is a non-steroidal estrogenic mycotoxin produced by several *Fusarium* species colonizing various cereals but primarily maize [79]. The structure of ZEA is shown in figure 8.

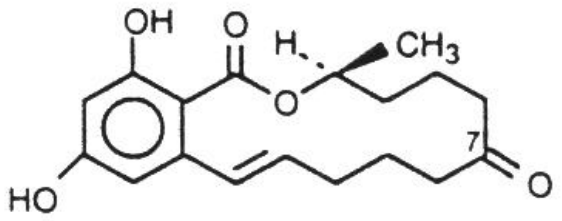

Figure 8. Structure of zearalenone [79]

Carcinogenicity (animal)

The NIH/NTP[a] considered the results of carcinogenicity assays in rats and mice to be positive evidence of carcinogenicity. In those studies, there was increased incidence of pituitary adenomas in both male and female animals, with progression to malignancy, as indicated by the presence of pituitary carcinomas in some of the animals [80].

Teratogenicity (animal)

Long et al. [81] investigated the effect of zearalenone, administered through the diet (1 mg/kg BW), on the development of swine embryos. From day 11 on degeneration of the embryos progressed mainly exhibited by disorganisation of the embryonic disk.

Immunotoxicity (animal)

In vitro studies of cytokine dysregulation help elucidate potential mechanisms of mycotoxin induced immunomodulation. Thus they will be important adjuncts to in vivo approaches for assessing immunotoxicity. In order to assess the immunotoxicity of zearalenone the murine thymoma cell line

[a] NIH/NTP = National Institutes of Health (USA)/National Toxicology Program

EL-4 was used. Both interleukin (IL)-2 and IL-5 levels were significantly elevated by ZEA exposure [51].

Genotoxicity (animal)

Zearalenone (ZEA) shows a DNA damaging effect in recombination tests with *Bacillus cereus*. Therefore investigations were undertaken to assess the effect of ZEA on DNA of female mice and rats after intraperitoneal (i.p.) or oral administration. After a single dose (2 mg/kg, i.p. or orally) DNA adducts were seen in kidney and liver of female mice. The total DNA adduct levels reached 114 ± 37 and 1393 ± 324 adducts/ 10^9 nucleotides respectively in kidney and liver after i.p. treatment and 548 ± 50 adducts/ 10^9 nucleotides in liver after oral treatment. In mice ovary DNA adducts appeared only after repeated doses (1mg/kg BW on days 1, 5, 7, 9 and 10). Some adducts were common to all organs, others were specific to a single organ. In contrast, no DNA adducts could be found in rat organs after i.p. treatment [79].

Haematotoxicity (animal)

In order to investigate the haematological effect of zearalenone female rats were given the substance intraperitoneally at levels of 1.5, 3 and 5 mg/kg. The platelet count decreased significantly dose dependently. Haemoglobin content was affected only at the 3 and 5 mg/kg doses and showed a significant increase [82].

Haematotoxicity (human)

Estrogens are able to influence the prostacyclin/thromboxane system. Prostacyclin has vasodilatory and antiaggregatory properties, while thromboxane is its counterpart showing vasoconstrictory and aggregatory activity. Since ZEA has estrogenic properties, Neuer et al. [83] tested the influence of ZEA on the prostacyclin/thromboxane system using human endothelial cells. ZEA stimulated prostacyclin production in low concentrations (10^{-7} and 10^{-8} M) and inhibited it at higher concentrations (10^{-5} M). No changes were observed in the thromboxane production.

Reproductive disorders (animal)

ZEA is a non-steroidal estrogenic mycotoxin with swine being particularly sensitive [84]. Hyperestrogenism, appearing when ZEA contamination of corn exceeds 1 ppm, shows different

characteristics dependent of the age of the swine. Only sexually mature boars are relatively insensitive to ZEA contamination [84-87]. When swine and mink were exposed to ZEA (2 and 20 ppm respectively) the breeding performance of swine was not affected although some hyperestrogenic effects were observed in the F1 piglets at 21 days of age. All the female mink exposed to ZEA mated, but only 25% whelped. Histological examination showed that the reproductive tracts of the ZEA treated mink were mildly to severely affected [88].

To investigate the estrogenic actions of ZEA on the reproductive organ system of rodents neonatal female mice were exposed to various doses of ZEA. Treatment resulted in inactivating effect on the reproductive organs of the animals and decreased serum levels of oestrogen [89].

Bioassays

By using the brine shrimp bioassay the concentration that causes 50% mortality (LC_{50}) has been determined at being 32.7 ppm [69].

Conclusive remarks

Zearalenone (ZEA), an estrogenic mycotoxin produced by various *Fusarium* species, shows two major adverse health effects in animals.

Not only has ZEA carcinogenic potential, as demonstrated by studies in the United States, but, due to its estrogenic nature, ZEA causes reproductive disorders in pigs. In mice ZEA affects ovary DNA, a phenomenon that might have effect on reproduction too.

Apart from researches on the prostacyclin/thromboxane system using human endothelial cells no studies were found describing effects caused by ZEA in humans.

4. Fumonisins

Introduction

Fumonisins were first found to be produced by *Fusarium (F.) moniliforme* in 1988 [90] and to a lesser extent by *F. proliferatum* and *F. anthophilum. F. subglutinans* is a non-consistent producer. Also other *Fusarium* species produce fumonisins.

Alternaria alternata f.sp. lycopersici produces a compound, AAL-toxin, which not only bears structural resemblance to fumonisin B_1 with respect to the main frame (see figure 9 and 10) but also produces similar effects as fumonisin B_1 [91 92].

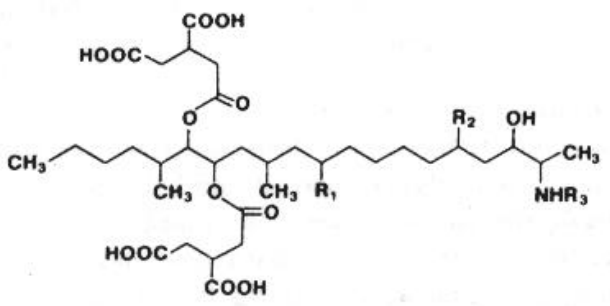

Figure 9. General structure of fumonisins [91]

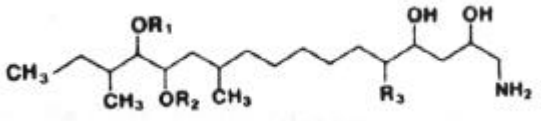

Figure 10. General structure of AAL toxins [91]

To date six fumonisins (see table 2) have been identified and purified, namely fumonisin A_1 and A_2, and fumonisin B_1, B_2, B_3 and B_4 [93]. The most important fumonisin with respect to health risks is fumonisin B_1, also being the fumonisin primarily discussed here.

The mode of action of fumonisins and AAL is mainly based on their structural resemblance to sphingoid bases, which are involved in membrane build-up. Fumonisins and AAL inhibit the

synthesis of sphingolipids, thus impairing membrane formation and cell viability. Although in the rest of this chapter primarily fumonisins are mentioned, it is imaginable that also AAL can be meant.

Table 2. Fumonisins (for R_1, R_2 and R_3 see figure 9)

Fumonisin	R_1	R_2	R_3
B_1	OH	OH	H
B_2	H	OH	H
B_3	OH	H	H
B_4	H	H	H
A_1	OH	OH	CH_3CO
A_2	H	OH	CH_3CO

Association with animal disease

Fumonisin B_1 (FB_1) is related to at least 2 diseases in livestock that have a high fatality rate, namely equine leukoencephalomalacia (ELEM) and porcine pulmonary oedema (PPE) [94]. Also FB_1 was found to be carcinogenic in the rat [95]. How the doses that cause natural disease relate to doses used in experimental conditions can be read from table 3.

Association with human disease

By circumstantial evidence FB_1 is believed to be the causative agent for oesophageal cancer in humans. In a few discrete areas around the world, Transkei and China, the high incidence of maize infected by *Fusarium* together with their mycotoxins, fumonisin B1 in particular, is correlated with a high incidence of oesophageal cancer [96]. In table 3 a comparison is made between the disease-causing intake of FB_1 and the "normal" conditions.

Carcinogenicity (animal)

Long term exposure to FB1 leads to carcinogenicity as shown by Gelderblom et al. [95], when rats were fed during 26 months a diet containing 50 mg FB1/kg feed. After 18 months the first signs of carcinomas in the liver (hepato-carcinomas) were visible in treated rats and not in control animals.

A recently concluded study within the framework of the National Toxicology Program of the National Institutes of Health (USA) found clear evidence of carcinogenic activity of FB_1 to male Fisher344/N rats and to female $B6C3F_1$ mice [97].

Table 3. Fumonisin doses in relation to pathological effects (copied from [98])

Exposure	Fumonisin concentration (ng/g feed)	Fumonisin intake (mg/kg BW[a]/day)
Natural outbreak of LEM (USA)	72,000	0.6 – 2.1
Experimental LEM in horses		1.25 – 4.0
Experimental Carcinogenesis[b] in rats	50,000	3.75
Person eating "healthy"[b] Transkeian corn	2,100	0.014
Person eating "moldy"[c] Transkeian corm	67,420	0.44

[a] BW = body weight
[b] based on a 200 g rat eating 15 gr feed per day
[c] baxed on 70-kg person eating 460 g corn per day

The mechanism by which FB_1 causes hepato-carcinogenicity in rats is still quite unknown but various investigators contribute to the elucidation of this enigma. Knasmüller et al. [25] found that FB_1 exposure caused chromosomal aberrations in rat hepatocytes at levels 6 to 7-fold higher than in unexposed cells. Such chromosomal aberrations may be an underlying condition for the occurrence of carcinogenesis. Martinez-Larranga et al. [99] investigated several metabolic functions in rat livers after repeated fumonisin exposure to assess their contribution to the development of hepato-carcinomas.

Cytotoxicity (animal)

Fumonisins and AAL resemble sphingoid bases, used for the build-up of membranes. Through their resemblance fumonisins and AAL are able to inhibit the synthesis of sphingolipids. The cellular uptake of certain compounds such as vitamins is able by the action of membrane anchored receptors. If, however, the build-up of the membrane is impaired by the action of fumonisins and AAL, also the uptake of vitamins, essential for certain cytosolic metabolism, is impaired, what may lead to cell death [92] [100].

Not only sphingoid based membranes suffer from exposure to fumonisin B1, long term incubation of Swiss 3T3 cells (fibroblasts) with fumonisin B1 disrupts cellular processes involving actin such as cytoskeleton formation [101]. Also in Swiss 3T3 fibroblasts it was found that fumonisin B1 acts like a mitogen and more specifically stimulates transiently the action of mitogen-activated protein kinase, a key enzyme in the signal transduction pathways activated by many mitogens [102].

Besides the kidney in rats, the liver is the target organ in rats and mice for chronic exposure to fumonisin B_1, as shown after a ninety day study by Voss et al. [103] and after a single intravenous dose by Lim et al. [104]. Their findings suggest that toxigenesis may be mediated by disruption of the *de novo* sphingolipid biosynthesis. Also another 4-week study by Voss et al. [105] supports the hypothesis that toxicity caused by fumonisin may result from altered sphingolipid synthesis.

The effect of fumonisin B1 exposure on sphingolipid biosynthesis in developing chick embryos has been evaluated by Zacharias et al. [106]. After administration of fumonisin into the yolk sac the incorporation of galactose and serine into embryonic sphingolipids was reduced about 70%, and sphinganine concentration increased dramatically. The inhibition of sphingolipid synthesis by fumonisin B_1 has been investigated by others too in different test systems, like cultured neurones by Merrill et al. [107].

Comparison of the cytotoxicity of fumonisins B, carried out with rat hepatocytes, learned that B_3 was the most cytotoxic followed by B_2 and B_1. In general the cytotoxic effect of fumonisins is low as can be derived from the CD_{50}[a], which is 2 mM for B_1 and 1 mM for B_2 [108].

[a] CD_{50} = concentration that causes death in 50% of the cells.

Cytotoxicity (human)

Primary human keratinocytes and human oesophageal epithelial cells were incubated with different concentrations of fumonisin B1. FB1 had no effect on both celltypes at concentrations up to 1 µM. The growth of keratinocytes and epithelial cells was inhibited by 42% and 75% respectively when exposed to 10 µM and 100 µM FB1 respectively [109].

Immunotoxicity (animal)

The influence of fumonisin on the immune system of pigs, and especially tumour necrosis factor alpha (TNF-a), was measured by feeding them fumonisin containing *Fusarium moniliforme* culture material. After oral administration the pigs not only showed signs of pulmonary oedema, a disease caused by fumonisin specific for pigs, but also increased levels of TNF-a. After intragastric administration both the signs for pulmonary oedema were fulminant and the TNF-a levels increased rapidly. Whereas the pig is an important test animal for the study of TNF mediated toxic shock, it is important to make sure the diet is free of fumonisin [110].

Macrophages produce nitric oxide (NO) which can be toxic to e.g. lymphocytes. Rotter and Oh [111] have shown that fumonisin B1 stimulates the NO-production and decreases protein concentration of murine macrophages (cell line RAW 264.7) dose dependently. The protein content served as marker for the cellular mass. The involvement of FB1 is difficult to explain because of the complexity of NO generation.

Neurotoxicity (animal)

Equine leukoencephalomalacia (ELEM) is caused by the consumption of maize contaminated with *Fusarium moniliforme* as was shown by Kriek et al. in 1981 [112]. The purification and structure elucidation of fumonisin B_1 made it possible to try and induce ELEM with this substance. Marasas et al. [113] found that intravenous administration of fumonisin B_1 to horses led to the emergence of symptoms of ELEM, establishing that indeed fumonisin B_1 is responsible for this equine disease. These findings were supported by the fact that oral administration of fumonisin B_1 to horses also led to symptoms of ELEM [114]. Rats exposed for 4 weeks to *Fusarium moniliforme* culture material showed changes in concentration of some neurotransmitters including serotonin. When rats were exposed to purified fumonisin B_1, no changes in neurotransmitters were not seen indicating that a toxin other than fumonisin is responsible for the changes seen after exposure to culture material [115].

Haemotoxicity (animal)

Low doses of fumonisin may, transiently, alter the concentration of several serum components. When broiler chicks were fed a diet containing 30 mg fumonisin B_1/kg feed for 6 days and were allowed to recover for 5 weeks concentration of several serum proteins were similar to those of control animals. When, however, similarly fed chicks were necropsied after 6 days of feeding fumonisin B_1 contaminated feed the concentration of serum proteins differed significantly from those of control animals [116]. Erythrocytes and abnormally shaped red cells were observed in the blood of broiler chicks on day 21 after hatch. The chicks had been fed *Fusarium proliferatum* culture material containing fumonisins B_1 and B_2 and moniliformin [117].

Pulmonary disorders / Respiratory disorders

In 1989/1990 34 mature swine died at two Georgia farms with symptoms and pathology indicating pulmonary oedema and hydrothorax. Both farms had purchased corn from the same local grain dryer. After research the feed appeared to be contaminated with *Fusarium moniliforme* and fumonisin contents ranged from 105 to 155 mg/kg. Experiments with pigs fed the same diet as the deceased pigs showed the same symptoms and resulted in the death through pulmonary oedema and hydrothorax as seen in the field cases [118].

Pulmonary oedema represents an acute effect from fumonisin B_1 in pigs. High incidental doses are necessary to invoke this disease. Becker et al. [119] have investigated the effects of non-lethal low doses of fumonisin B_1 on lactating sow and pigs. When fed a diet containing 100 ppm fumonisin B_1 for 17 days no detectable amounts of fumonisin B_1 were found in the sow's milk, no evidence of toxicosis could be found and no significant effect on T-lymphocyte function in sows and pigs could be detected. Also higher environmental temperatures did not enhance toxic effects of fumonisin B_1.

Growth disorders (animal)

Broiler chicks were fed a diet containing 300 mg fumonisin B_1/kg of diet from day of hatch to day 21 of age. BW[a] gains were reduced by 18 – 20% after this period. A diet containing fumonisin B_1 and T-2 toxin (300 mg and 5 mg per kg of diet respectively) reduced BW gain by 32%. According to the authors the increase in percentage reduction of BW gain is to be attributed to additive toxicity instead of synergism of the toxins. Also, the combination-diet of fumonisin B_1 and T-2 toxin caused a mortality of 15% compared to the control group where no animal was lost during the study [39].

In turkey poults, fed a diet containing 300 mg fumonisin B_1 /kg diet too, BW gain during the first three weeks of life was reduced by 24 – 30% [62 78]. Contrary to the findings in broiler chicks [24], mortality was not raised in turkey poults [78]. Bermudez et al. [120], however, found that turkey poults fed a diet containing 200 mg fumonisin B_1/kg feed hardly showed a decrease in BW gain.

In barrows 28 day exposure to fumonisin B_1 (100 mg/kg feed) led to reduced weight gain as well as changes in haematological parameters, decreases and increases dependent on the investigated compound [28]. In general, growth problems in pigs become a problem at prolonged exposure to diets containing 1 – 10 ppm of fumonisin [121].

Reproductive disorders (animal)

Diets contaminated with *Fusarium moniliforme* culture material containing 0, 1, 10 or 55 ppm fumonisin B_1 were fed to male and female rats beginning 9 and 2 weeks before mating and continuing throughout mating, gestation and lactating phases. Despite increased sphinganine to sphingosine ratios in the dams of the 55 ppm group no reproductive effects were measured, indicating that culture material and by inference FB_1 have no influence on reproduction in rats [122].

Cardiovascular effects (animal)

Male pigs were fed a diet containing 20 mg/kg fumonisin-containing culture material for 7 days. Maximal rate of change of left ventricular pressure, heart rate, cardiac output and mean aortic pressure were significantly decreased after this feeding period, while mean pulmonary artery pressure, pulmonary vascular resistance were significantly increased. The animals also suffered from decreases in O_2 tension in both arterial and mixed venous blood and systemic oxygen delivery, and increases in oxygen consumption and oxygen extraction ratio [123].

Conclusive remarks

The disorders mentioned here give a good picture to what fumonisins in general and fumonisin B_1 in particular are capable. Most noticeable is the carcinogenicity to humans as demonstrated by the cases of eosophageal cancer in Transkei and China. Although knowledge is increasing rapidly, many of the exact actions and much of the structure/action relationship is still to be clarified. Fumonisins are structurally similar to sphingoid bases (such as sphingosine and sphinganine) and several fumonisins have been shown to block the *de novo* synthesis of sphingolipids through specific inhibition of enzymes, thus affecting the concentrations of sphingosine (So) and sphinganine (Sa). This led to the understanding that the Sa/So ratio might act as an indicator of exposure to fumonisins [124].

Growth problems in pigs occur mainly after prolonged exposure to fumonisin B1. Also prolonged exposure to fumonisin B_1 (FB_1) may lead to accumulation of FB1 in the animal, with a preference for liver and kidney. When such animals are used for food production man could be exposed to FB1 through the consumption of contaminated meat. Another problem arising from prolonged exposure is of economical nature namely the decreased weight gain of the pigs leading to lower slaughter weight and less income for the owner [121].

Apart from its carcinogenicity many of the implications for human health are still unknown.

[a] BW = body weight

5. Aflatoxins

Introduction

Aflatoxins are a group of closely related mycotoxins that are widely distributed in nature [125]. They are produced primarily by some strains of *Aspergillus (A.) flavus* and most, if not all, strains of *A. parasiticus*, plus a related species, *A. nomius* [13]. Next to aflatoxin B_1 the following aflatoxins (AF's) are the most important: AFB_2, AFG_1 and AFG_2, the hydroxylated forms of AFB_1 and AFB_2, namely AFM_1 and AFM_2 respectively, and the 8,9-hydrated forms of AFB_1 and AFG_1, namely AFB_{2a} and AFG_{2a} respectively [125]. The structure of these most important aflatoxins can be found in figure 11.

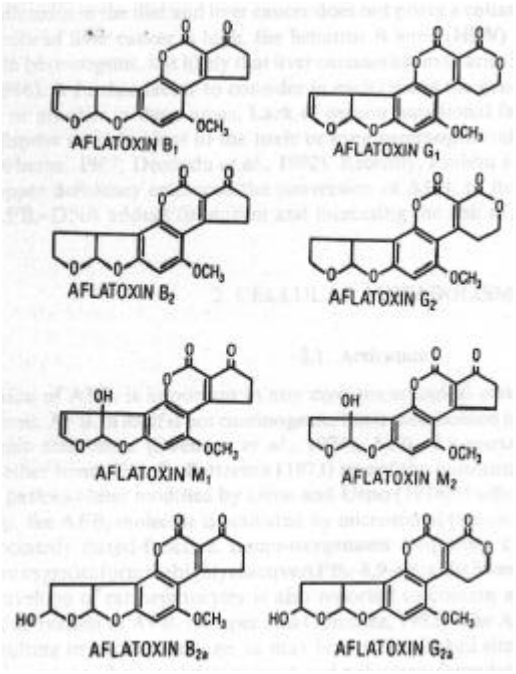

Figure 11. Structure of aflatoxins B_1, B_2, G_1, G_2, M_1, M_2, B_{2a} and G_{2a} [125].

Aflatoxin was first discovered in 1959 because of its acute toxicity and it became apparent during chronic toxicity studies in the early 1960s that aflatoxin B_1 was also a potent carcinogen in at least some animal species [126].

Because of their high toxicity the group of the aflatoxins and their toxicological effects is studied closely, as demonstrated by various extensive review articles [13 125-129]. Needless to say that this

literature review on the biological activity of aflatoxins is far from complete, but is only meant to list some of the toxic potentials of aflatoxins.

Carcinogenicity (human)

Aflatoxins are carcinogenic, as expressed by the International Agency for Research on Cancer, which concluded in 1987 that there is sufficient evidence for the carcinogenicity of aflatoxins to humans [94].

Carcinogenicity (animal)

The liver is the primary target in different animal species (hepato-carcinogenic). Of the aflatoxins AFB1 is the most carcinogenic, the most occurring, and the most potent hepatocarcinogen to rats and mice. AFM_1, excreted in milk of lactating cows, has toxic properties similar to those of AFB_1 [130]. Aflatoxins in general and AFB1 in particular are known to cause cancer in different animal species as shown in table 4 [127].

In addition to being present in foodstuffs, AFB_1 can contaminate respirable grain dust and thus the respiratory system is a potential target for carcinogenesis [131]. To study the potential effects of AFB_1 in the upper airways, the metabolism of AFB_1 was investigated in tracheal cultures and purified tracheal microsomes from rabbit, hamster and rat. It was concluded that rabbit upper airway epithelium contains metabolic activity primarily involved in AFB_1 activation, whereas AFB_1 detoxification pathways predominate in the hamster [132].

Table 4. Carcinogenicity of aflatoxin B1, depicting dosage and period of oral exposure [127]

Species	Dose per kg body weight	Period of exposure	Tumor frequency (%)
Duck	30 μg	14 months	8/11 (72%)
Trout	8 μg	1 year	27/65 (40%)
Monkey	100—800 mg	> 2 years	3/42 (7%)
Rat	100 μg	54—88 weeks	28/28 (100%)
Mice	150 μg	80 weeks	0/60 (0%)

Modulation of carcinogenesis by dietary components may play a significant role in the aetiology of human cancer. Fisher 344 rats were fed different diets prior to AFB1 administration. A choline-methionine deficient (CMD) diet resulted in no obvious time or dose dependent response to toxin treatment as far as serum and pathology data are concerned. Whereas a single dose of AFB_1 during a nutritionally complete amino acid defined basal (CMS) diet resulted in changed serum concentrations of different serum enzymes and serum constituents. In CMS fed rats a NOEL (no observed effect level) of 100 µg/kg was found. These data indicate that diet composition is of importance to the susceptibility of the liver to AFB_1 [133].

Mutagenicity

To assess mutagenic properties of different aflatoxins a modification of the Ames test with *Salmonella typhimurium* (see chapter "Bioassays") was used. AFM_1 and AFB_1 had specific mutagenic activities of 13 and 121 revertants/ng respectively [134].

Cytotoxicity (animal)

The MTT-cell culture assay is a means of determining the cytotoxicity of various substances ([135], see chapter "Bioassays"). AFB1was used in the MTT test using three different cell cultures, namely swine kidney (SK) cells, Madin-Darby canine kidney (MDCK) cells and Helene-Langer (HeLa) cells. AFB1 was cytotoxic at the following levels: > 50 µ/ml to SK cells, > 200 µg/ml to MDCK cells and 25 µg/ml to HeLa cells [136].

Immunotoxicity (animal)

The influence of different mycotoxins on the production of Interleukin (IL-)-2 and IL-5 was measured by incubating the thymoma cell line EL-4 with various concentrations of the different toxins. AFB_1 did not affect IL-2 or IL-5 production at concentrations up to 10 µg/ml [51].

Respiratory tract cilia represent one of the most important biological barriers between human organisms and the environment. AFB_1 inhibits movement after 6 days at a concentration of 30 µg/l. AB_2 and AFM_1 had almost the same effectiveness as AFB_1. Much less ciliostatically effective in vitro were AFG_1 and AFG_2 [60].

Immunotoxicity (human)

Aflatoxins B_1, B_2, G_1 and G_2 have proven to have significant mitogenic effect on human T_4 lymphocytes, while no adverse reactions were seen upon administration of doses of 10, 50 and 100 µg on a modest clinical basis to volunteers [137].

AFB_1 induced inhibition of phagocytosis of Kuppfer cells at concentrations as low as 0.01 pg/ml. The metabolites AFQ_1 and AFM_1 had similar effect on phagocytosis, but were two- to ten-fold less potent than AFB_1 [138].

When rat bone marrow cells were exposed to AFB_1 at a concentration of 1 mg/kg body weight (approximately $^1/_5$ of the LD_{50}[a] for young male rats) granulopoietic toxicity was observed and changes in the production of humoral regulatory factors dealing with the granulopoietic developmental pathway. CFU-GM[b] was transiently suppressed by AFB_1, the peak in granulopoietic activity was preceded in time by an increased CSA[c] and IL[d]-1 formation, and elevated IL-2 synthesis and increased T-cell activation paralleled the peak in granulopoietic activity [139].

Growth disorders (animal)

When broiler chicks were fed a diet containing 3.5 mg AFB_1/kg, the BW gain was reduced by 13% [140]. A similar figure for reduced BW gain was found by Huff et al. [141]. However, when mallard ducklings were fed diets containing 12 ppb or 33 ppb AF no significant differences in final BW compared to non-exposed controls were observed [63].

That different species react different to the same mycotoxin is also demonstrated by studies regarding growth effects in different bird species. After exposure to 2.5 and 5 ppm AFB_1 BW gain of bobwhite quail was after two weeks significantly decreased, whereas only the highest level of AFB_1 decreased BW gain in the Japanese quail, and even to a much lesser extent than in bobwhites [65]. Ringneck pheasants proved to be even more sensitive to AFB_1 exposure, the lowest level applied (1.25 ppm) decreased the BW gain already after one week of exposure [64].

[a] LD_{50} = dose that is lethal to 50% of the animals
[b] CFU-GM = granulocyte-monocyte colony-forming unit
[c] CSA = coclony-stimulating activity
[d] IL = interleukin

Allergenicity (animal)

During allergic reaction when the allergen contacts the cell bound IgE histamine and other mediators present in mast cells are released. AFB_1, AFG_1 and AFB_2 prepared from *Aspergillus* species cultured from airborne spores during harvest induced in rabbits an increase in histamine level 18 hours after injection. Therefore it was concluded that these toxins can also act as allergens [142].

Conclusive remarks

Aflatoxins in general, and aflatoxin B_1 (AFB_1) in particular, are mycotoxins produced by various species of *Aspergillus* and *Penicillium*. Since their discovery they have been studied extensively and many effects have been elucidated. Apart from the most prominent property, the carcinogenicity of AFB_1, immunotoxic, haematotoxic, and growth effects have been observed after administration of aflatoxins. Many different animal species can be affected by aflatoxins. However, extrapolation of effects from one species to another is hindered by inter- and intraspecies susceptibility differences [143] [144], thus making extrapolation of effects found by animals to possible effects in humans invalid.

6. Ochratoxin A

Introduction

Ochratoxin A (OTA), first described in 1965 as a toxic metabolite of *Aspergillus ochraceus*, is a mycotoxin produced by *Penicillium verrucosum* in temperate or cold climates and a number of species of *Aspergillus* in warmer and tropical parts of the world. *Penicillium verrucosum* is especially associated with stored cereals and is very common in Northern European countries and Canada. The structure of ochratoxin A is depicted in figure 12.

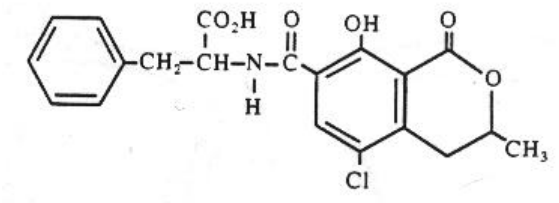

Figure 12. Structure of ochratoxin A [145]

Although under experimental conditions numerous derivatives of ochratoxin have been detected, only OTA and, extremely rarely, ochratoxin B (OTB) have been found to occur naturally in moulded plant products. OTA has been reported as naturally occurring in almost all cereals including corn, barley, wheat, sorghum, rye, oats and rice. OTA has been found to occur in other food commodities such as coffee and in meat and meat products produced from animals fed on contaminated feed. Post harvest formation of OTA is usually regarded as the predominant factor in contamination of food and feed. Worth mentioning is the fact that citrinin is often found together with OTA in naturally contaminated cereals [13].

Due to its occurrence in such a wide range of food commodities, the presence of OTA in human blood has been suggested as an indicator for indirect assessment of exposure [13].

Association with animal disease

Most important animal disease in relation to OTA is mycotoxic porcine nephropathy. The disease has been regularly reported in studies in Denmark since discovery 50 years ago and is recognised as an endemic disease in several northern and central European countries [146].

Association with human disease

Circumstantial evidence linked ochratoxin A to Balkan Endemic Nephropathy (BEN). BEN is characterised by renal failure and atrophy of the kidney with multiple clinical features. Endemic areas tend to be rural and most sufferers are from an agricultural background. Within two years after having acquired the disease ca. 50% of the sufferers die. Because BEN is almost exclusively encountered in individuals between the ages of 35 and 55, it is believed that prolonged exposure to a causal agent is a prerequisite for acquiring the disease [23]. Patients with the disease also have a high risk of developing urinary system tumours [80].

Ochratoxin A exposure is also associated with a significant higher incidence of chronic interstitial nephritis (CIN) in North Africa (Tunisia). The similarity between the clinical symptoms of BEN and CIN warranted a closer scrutiny for the possible role of OTA. 95% of the people in Tunisia suffering from nephropathy are OTA positive with blood concentrations higher than 90 ng/ml in several cases [130 147].

Carcinogenicity (animal)

In an NIH/NTP[a] study F344/N rats were exposed to ochratoxin A for two years. At levels as low as 70 µg/kg BW/day, in males, a high incidence of renal adenomas and carcinomas were found together with a high incidence of metastases. Female rats were found to be less sensitive, but at 210 µg/kg BW a dose related increase in renal adenomas was found, and renal carcinomas were also present [148].

Several authors have investigated the carcinogenic effect of OTA in laboratory animals, especially mice. Long term exposure to OTA resulted in renal tumours. As mentioned in the introduction of this chapter citrinin is often encountered in the presence of OTA. The effect of this combined occurrence has been demonstrated by investigations using both toxins separately and in combination. Citrinin alone does not induce renal tumours, exposure to OTA produces tumours in 6 out of 20 mice and exposure to OTA and citrinin together leads to tumours in 10 out of 18 mice. So citrinin appears to have an additional toxicity only in conjunction with OTA [146].

[a] NIH/NTP = National Institutes of Health (USA)/National Toxicology Program

Carcinogenicity (human)

Since 1993 the International Agency for Research on Cancer (IARC) has classified OTA as a possible human carcinogen based on sufficient evidence in experimental animal studies and inadequate evidence in humans[149].

Cytotoxicity (animal)

OTA induces unscheduled DNA synthesis (UDS) in rat hepatocytes in a very narrow concentration range. At 750 nanoMolar (nM) a weak and at 1 µM a marked induction was observed. Concentrations < 750 nM had no effect, while concentration > 1 µM were cytotoxic. Similar experiments were performed using porcine urinary bladder epithelial cells (PUBEC), and similar results were found at slightly different concentrations. Between 250 and 1000 nM dose dependent UDS was observed, while concentrations < 250 nM had no effect and concentrations > 1 µM were cytotoxic [150].

The cytotoxic effect of OTA is mainly based on the structural analogy with phenylalanine (Phe), inhibiting protein synthesis by competition with Phe in the Phe-tRNA aminoacylation reaction. Treatment of Vero-cells with aspartame (L-aspartyl-L-phenylalanine methyl ester) led to a decrease in protein inhibition due to the exposure to OTA. Also, leakage of certain enzymes from the cells into the culture medium, a mechanism that is OTA-induced, was prevented by the aspartame treatment [151].

Immunotoxicity (animal)

To measure the effect of prenatal exposure to OTA on the immune system, Balb/c dams were exposed to doses of 30 or 200 µg/kg feed before and during gestation. At birth, pups were cross-fostered to non-exposed dams. In the offspring of the high dose group (200 µg/kg diet) at days 14 and 28 a decrease in percentages of splenic $CD4^+$ and $CD8^+$ cells was observed but no significant alterations in the absolute numbers of splenocytes. In the thymus, a relative and absolute increase of $CD4^+$ numbers was seen on day 14 and an increase of $CD4^+$, $CD8^+$ and $CD4^+CD8^+$ numbers at day 28. Functional changes to splenocytes were not observed, which lead to the conclusion that low level exposure to OTA alters the relative and absolute numbers of lymphocyte subpopulations in lymphoid organs but does not affect the immune functions [152].

A single dose exposure of Balb/c dams to OTA (500 µg/kg bodyweight) on day 16 of gestation resulted in decreased proliferation of splenic and thymocyte lymphocytes in response to mitogens in the pups at days 15 of age. Lower percentages of mature CD4+ cells and higher percentages of immature CD4+CD8+ double positive cells were found in the exposed pups. A single exposure of the dams on day 10 post-partum significantly increased the proliferative responsiveness of lymphocytes in the offspring when stimulated with B or T cell mitogens 3 days after exposure. With these experiments is has been shown that the time of exposure significantly influences the immunotoxic effects of OTA in the developing immune system in rodents [153].

Müller et al. [154] found that in a mouse model OTA has a non-selective suppressive effect on various immune and defence systems. Not only do weight depression, lymphopenia, neutrophilia and eosinophilia occur, also antibody production and phagocytosis become suppressed. And moreover, immunised animals show a lower survival rate after experimental infection with *Pasteurella multocida*.

The influence of exposure to ochratoxin on the production of various interleukins was investigated by incubating thymoma cells (EL-4) with different concentrations of OTA. Both concentrations of 5 and 10 µg/ml OTA markedly increase the production of interleukin (IL-)-2 while production of IL-5 was significantly decreased [51].

Genotoxicity (animal)

Exposure of mice to a single dose of OTA (2.5 mg/kg) through gastric intubation led to modifications in DNA in kidney, liver and spleen 24 hours after administration. Although in all organs modifications were found the levels of changes differed in a tissue dependent manner with the kidney and liver being the most sensitive organs [155].

The kidney represents one of the main targets for OTA. The mode of action is still under investigation. Schramek et al. [156] investigated part of the intracellular signal transduction by using Madin-Darby canine kidney cells and found, under the influence of OTA, epithelial dedifferentiation resembling alkali-treatment, mainly due to changes in signal transduction molecules.

Renal cortex explants from male swine were used to study the effect of OTA on a number of parameters. Macromolecule biosynthesis, i.c. DNA-synthesis, was inhibited from a concentration of 0.001 milliMolar (mM) on, protein synthesis was inhibited from a concentration of 0.01 mM on. RNA synthesis (60 % inhibition at 1 mM) was affected the least of the macromolecules studied [157].

In some birds too the kidney is one of the major sensitive organs for OTA. It has been shown that kidney weight in 3-week-old Japanese quail increased dose dependent after exposure to OTA (53). Also the weight of the kidney of 3-week-old pheasants increases dose dependent after exposure to OTA [64].

Genotoxicity (human)

Manolov et al. [158] found that in lymphocytes from BEN patients chromosomal anomalies, like monosomic and polysomic forms of chromosome X in female patients, were present. Similar symptoms were found in lymphocytes from healthy individuals exposed to OTA, supporting the hypothesis that OTA is involved in the pathogenesis of BEN.

Maaroufi et al. [159] proved in renal biopsies from patients suffering from chronic interstitial nephropathy that OTA binds to DNA (more specific to guanine in DNA) thus rendering the DNA less active and thus affecting the renal function.

Neurotoxicity (animal)

Foetal rat telencephalon cells were exposed during the early developmental period and during advanced maturation to different concentrations of OTA. A ten-day treatment at 50 nM[a] caused general cytotoxicity in both immature and mature cells. A 24-hour treatment resulted in dose dependent decrease in protein synthesis with IC_{50}[b] values of 25 and 33 nM for immature and mature cells respectively [160].

Investigations using embryonic chick brain and neural retina cell cultures have revealed a neurotoxic effect for OTA. Regarding the fact that in vivo OTA is nearly completely bound to serum proteins, the same cell cultures were exposed to OTA bound to BSA. IC5 and IC50 values increased, 15-fold for brain and 32-fold for meningeal cell cultures. Although OTA is a structural analogue of phenylalanine, in these cell cultures no competition could be seen with pehnylalanine dependent processes [161].

Possible alterations in the brain of female rats were examined following subchronic application of OTA (1.5 ppm per day). Main targets of research were a number of soluble and membrane bound enzymes in the cerebral cortex, cerebellum and hippocampus. Physiologically significant alterations in

[a] nM = nanoMolar

[b] IC_{50} = concentration at which 50% inhibition is noticed

the activity of enzymes tested were found. The changes were found to be time-dependent and regionally selective [162].

Nephrotoxicity (animal)

OTA has been reported to induce lipid peroxidation, which might play a role in the nephrotoxicity of OTA. To confirm this report rats were subcutaneously injected with superoxide dismutase (SOD) and catalase every 48 hours for three weeks, the first injection taking place 1 hour before OTA exposure (289 µg/kg body weight every 48 hrs). SOD and catalase prevented most of the nephrotoxic effects of OTA, indicating that superoxide radicals and hydrogen peroxide are very likely to be involved in the damaging processes of OTA [163].

To study the effect of OTA on the protein endocytosis of kidney cells, proximal tubule-derived opossum kidney cells were exposed to OTA during the 15 minute period in which uptake of albumin was studied. This exposure protocol had no effect on the albumin endocytosis. When however the cells were pre-incubated for 24 hours transport capacity and specific binding of albumin to apical cell surface were reduced. Furthermore the re-exocytosis of albumin was significantly greater in OTA-treated cells. These experiments indicate that proteinuria is part of the effects caused to the renal system by OTA exposure [164].

Haematotoxicity (animal)

Exposure of mice to OTA (5 mg/kg daily for 6 weeks) induced hypocellularity in the bone marrow, leading to diminished numbers of precursors for erythrocytes, white blood cells and megakaryocytes. Exposure to higher doses also leads to bone marrow hypocellularity with a maximum decrease in granulocyte-monocyte progenitors of 25%, leading to the conclusion that OTA is myelotoxic but not cytotoxic [129].

Pulmonary disorders / Respiratory disorders

Respiratory tract cilia represent one of the most important biological barriers between human organisms and the environment. OTA inhibits movement after 6 days at a concentration of 1.2 mg/l and after 2 days at a concentration of 20 mg/l [60].

Growth disorders (animal)

Turkey poults fed a diet containing 3 mg OTA/kg showed an 8% decrease in BW[a] gain compared to non-treated poults. In combination with fumonisin B1, which decreases BW gain with 30%, OTA enhances the fumonisin effect by 7%, leading to a decrease of 37% [78].

Broiler chicks fed on a diet containing 3 mg OTA/kg showed a significant decrease or BW gain while the relative weight of several organs (liver, kidney, spleen, pancreas) increased [165].

Reproductive disorders (animal)

The finding of OTA in testicles of laboratory animals led to investigations concerning possible adverse effect of OTA on rat testis. Possible impairment of spermatogenesis was found, seemingly to be due to an earlier modification of the androgen status, especially the testosterone concentration in the testis [166].

Conclusive remarks

Ochratoxin A (OTA), a mycotoxin produced mainly by *Penicillium verrucosum* (in the temperate climate zones) and several *Aspergillus* species (in the warmer climate zones), is associated with adverse health effects in humans, namely balkan endemic nephropathy and chronic interstitial nephritis. Together with the facts that OTA is considered a possible human carcinogen [167], that through accumulation OTA remains prolonged in tissues, that OTA affects the immunesystem, is cyto- and geno- and neurotoxic, and that OTA passes unchanged through the food chain [125 168], it is obvious that OTA is a very potent mycotoxin. Although sofar OTA involvement in human disease seems to be restricted to effects in the kidney, other organs may be affected too. Although many effects are the result of prolonged exposure to or accumulation of OTA, acute toxicity also plays an important role, with susceptibility varying from species to species (see table 5, page 61).

[a] BW = body weight

Table 5. Acute toxicity of ochratoxin A (copied from [153])

Animal	LD_{50}[a] (mg/kg body weight)	Route of administration
Mouse, female	22.00	ip[b]
Rat, male	30.3	oral
Rat, female	21.4	oral
Rat, male	28.00	oral
Rat, male	12.6	ip
Rat, female	14.3	ip
Guinea pig, male	9.1	oral
Guinea pig, female	8.1	oral
White leghorn	3.4	oral
Turkey	5.9	oral
Japanese quail	16.5	oral
Rainbow trout	4.7	ip
Beagle dog, male	<9 (total dose)	oral
Pig, female	<6 (total dose)	oral

[a] LD_{50} = dose lethal to 50% of the animals used
[b] ip = intraperitoneal

7. Citrinin

Introduction

Citrinin, the structure of which has some analogy with ochratoxin A and is shown in figure 13, is a metabolite of toxic strains of *Penicillium* and *Aspergillus* species and has first been described in 1931.

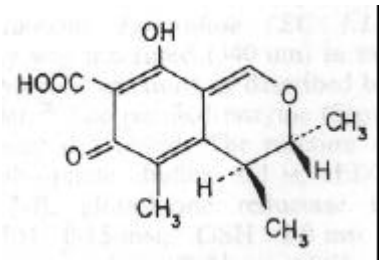

Figure 13. Structure of citrinin [169]

Citrinin is a well-known contaminant of various cereals, nuts, apple juice and cheese [169]. Citrinin is often encountered together with ochratoxin A [13] which finding has led to investigations regarding additive toxicity or synergism between these two toxins [170]. Although other lesions due to citrinin have been observed in other tissues, the kidney appears to be the primary site of action of citrinin.

Carcinogenicity (animal)

A long term feeding study with 1 gr citrinin per kg of diet utilizing male Fisher 344 rats for 80 weeks, led to massive tumours in the kidneys of all treated animals. These tumours were benign and were classified as clear-cell adenomas [171]. This led to the IARC conclusion that there is limited evidence for the carcinogenicity of citrinin to experimental animals [172]

Mutagenicity

Citrinin is able to cause single-strand breaks in the DNA of *E.coli* at a concentration of 100 µg/ml. In order to investigate whether such DNA damage led to mutagenicity, Brakhage et al. employed a reversion test using an amber mutant of phage M_{13}. They could show, with 50 µg citrinin/ml at pH 6.0, a low but significant increase of reversion of the phage. DNA sequence of 112 revertants

showed in all cases single base mutations in the former amber codon (positions 3066-3068). In some cases a second mutation 50 bases upstream was observed [173].

Nephrotoxicity (animal)

Rat renal proximal tubules were used to investigate the effect of citrinin exposure. Viability of the tubules depended on time of exposure and dose and time and dose dependent changes in the oxygen consumption were observed [174].

Baby hamster kidney (BHK-21) cells were used to study the cytotoxic effect of citrinin. Besides changes in the adherence of the cells to the culture bottles, morphological changes were found by microscopy: cells were no longer elongated and flat but under the influence of citrinin became swollen and round. This swelling of cells led to cell death. These effects were dependent on the toxin concentration as well as on the exposure time [175].

BHK-21 cells were also used to study the effect of citrinin more in detail, namely on energy production along the respiratory chain and on the glycolytic lactate production. Citrinin inhibited oxygen consumption, reducing the respiratory rate with 39%, and disabled the lactate production. The perturbations in BHK-21 cells caused by citrinin are due to alterations in mitochondrial function and in the glycolytic anaerobic pathway [176].

Knowing that citrinin is able to permeate membranes and hit the mitochondria, giving rise to its toxic effects, Ribeiro et al. [169] investigated the mode of action of citrinin in detail. It was established that citrinin modifies the antioxidant enzymatic defences of cells through the inhibition of glutathione-reductase and transhydrogenase. In this way the toxin increases the generation of reactive oxygen species, stimulating the production of superoxide anion in the respiratory chain, which eventually leads to cell death.

Besides effects on the respiratory chain in cells citrinin also affects the transport of calcium ions. Citrinin promotes a dose dependent decrease in the velocity and in the total capacity of Ca^{2+} uptake in mitochondria [177]. For these studies BHK-21 cells were used.

Mitochondria from rat kidney cortex and rat liver were used to further investigate the action of citrinin on these cell organelle. The results suggested that the toxin interferes with the mitochondrial membrane fluidity [178].

Bioassays

A useful method to investigate the cytotoxicity of mycotoxins is provided by the MTT-cell culture assay. Mehylthiazoltetrazolium (MTT) is converted by mitochondrial dehydrogenases of metabolically active cells to an insoluble purple formazan product, which is then solubilised with dimethylsulfoxide. The optical density of this homogeneous solution is suitable for precise spectrophotometric measurement. Citrinin causes toxic effects in SK-cells starting at a concentration of > 50 µg/ml, as measured by this assay [136].

Conclusive remarks

Citrinin is a toxic metabolite of various *Penicillium* and *Aspergillus* species.

The interest for citrinin is, like the interest for diacetoxyscirpenol (see chapter 2.5), triggered by its conjunctive occurrence with other mycotoxins, mainly ochratoxin A. The action of citrinin is, as far as known, primarily directed against the animal kidney. For any involvement in effects in humans no indications were found

8. Bioassays

In several previous chapters bioassays have been mentioned already. None of these bioassays is specific for the mycotoxin the chapter deals with. Usually generally applicable bioassays have been mentioned in connection to a specific mycotoxin.
Most of the previously mentioned bioassays will be discussed here again. This chapter is meant to give an overview of the most important bioassays used in combination with research in mycotoxins.

The term bioassay can be used in different manners; 1) an assay using a biological system of any kind intended to investigate whether exposure to a compound/substance has any effect on the biological system (general bioassays, for example, the use of a bioassay to investigate whether a certain fungal strain produces toxic metabolites), and 2) an assay using a biological system intended to investigate whether exposure to a compound/substance leads to a previously specified effect (effect specific bioassays, for example, the use of a biological system to investigate whether fungal metabolites have immunotoxic or immunomodulating potential).
In this chapter several general bioassays will be highlighted and a number of specific bioassays

8.1 General bioassays

Although most of the tests mentioned here are tests for cytotoxicity, it was deemed preferable to cite the next tests as being “general bioassays”, because their use is so universal and “cytotoxicity” is such a broad concept.

Brine shrimp test [179].

This test is described as a cytotoxicity test. Brine shrimp larvae (*Artemia salina*) are exposed to a certain substance to investigate whether this substance is able to cause immotility or even death of the shrimps. By using serial dilutions dose dependency can be studied.

Army worm larvae [180].

A test specific for the effect of T-2 toxin on BW[a] gain is described using a specific insect species. Larvae are fed on a diet containing 10-fold dilutions of T-2 toxin for 7 days. According to the authors similar tests using different insect species have been described for other mycotoxins.

Mosquito larvae [181].

This test is meant to assess general toxicity, and more specific the LC_{50}[b] of a compound. Mosquito larvae (*Aedes aegypti*) are exposed to 10-fold concentrations of the compound of interest to establish 0 and 100% response limits. Next, narrower ranges of the compound of interest are tested to establish the 50% response dose.

Chick and duck embryos [182].

The sensitivity for various toxic substances on embryos of chicks and ducks varies with the time of administration. It has been found that 10-day-old chick embryos and 13-day-old duck embryos are the most sensitive for pesticides, with duck embryos providing less variable results. The test might also be useful for the screening on toxicity of mycotoxins. This test screens for general toxicity, because the only criterium for effectiveness of the toxic substances is survival.

Rabbit skin sensitivity test [183].

T-2 toxin causes skin reactions, and more specific erythema, edema and necrosis, in rabbits. Control solutions, T-2 toxin standard solutions and sample solutions (extracts of corn suspected for the presence of T-2 toxin) are applied simultaneously to closely clipped rabbit skin. The reactivity of sample solutions is compared to that of standard solutions to give an answer to the T-2 content of the sample solutions.

[a] BW = body weight
[b] LC_{50} = 50% lethal concentration = concentration needed to induce 50 % mortality

Chick embryo toxicity screening test (CHEST)

This test is mainly described as a method to aid in the isolation of unknown (myco-)toxins, a biological assay to monitor the progress from initial identification of the toxic source, through purification of the extracts, to the establishment of relative toxicity of the purified compound. Various different designs of the test are available, and even the Association of Official Analytical Chemists (AOAC) has a recommended protocol. Prelusky et al. [184] describe a design that is simple and practical to carry out for routinely predicting the toxic potential of isolated metabolites.

MTT-cell culture test

This test screens for cytotoxicity. Cells (lymphocytes for example) are incubated with the tetrazolium salt MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]. Living cells are able to cleave MTT yielding MTT formazan, a dark blue product. MTT formazan, which is insoluble in water, is solubilized with DMSO. Absorbance readings determine the amount of MTT-formazan, which is proportional to the number of living cells. Different cell lines can be used, animal as well as human [116] [185]. This test is not specific for a certain mycotoxin, nor for a certain type of effect.

8.2 Effect specific bioassays

Mutagenicity / genotoxicity

Ames test with *Salmonella typhimurium* [186]

The principle of this test system is based on various mutations in bacterial tester strains, giving them certain properties. Mutagenicity of a compound is expressed by altered properties of the tester strains compared to controls. Over the years the test system has been used for the evaluation of numerous different chemicals, and the test system has been adapted to improve its performance.

Bioluminescence test

This is a test to screen compounds for mutagenicity or genotoxicity test as the afore mentioned Ames test. It employs dark mutants of luminous bacteria (*Vibrio fischeri*) to determine the ability of the tested agents to restore luminescence by inducing mutation. It has been described by Ulitzur et al. [187] and has been commercialized as the Mutatox® test [188].

Cytotoxicity

Bromodeoxyuridine (BrdU) incorporation

Some mycotoxins induce cell proliferation during which process the synthesis of new DNA is a major action. By monitoring the amount of bromodeoxyuridine (BrdU), an analogue for one of the DNA-bases, with the aid of an enzymatic-colorimetric assay the measure of incorporation into newly synthesized DNA can be determined as a measure for cell proliferation [189].

In vitro reticulocyte lysate assay [190]

Some mycotoxins, like diacetoxyscirpenol and fusarenon X, produce adverse effects on the protein synthesis system. For the investigation of this phenomenon the protein synthesizing system from rabbit reticulocytes can be used, as described in this article.

Mammalian tissue cultures [191]

Not only for assessing general cytotoxicity but also for the assessment of adverse effects on the synthesis of proteins and DNA human fibroblasts can be used. The method has been applied for the mycotoxins T-2 and T-2 tetraol, but other substances can also be tested for toxicity with this system. Also, cultures from different human cells can be used for assessing specific toxicity.

9. Discussion and conclusion

Risk assessment in RIVM-project 257852 entitled, Exposure to fungi and fungal products and the concurrent risk for the public health, will focus mainly on exposure to (toxic metabolites of) moulds in cereals and grain products. A previous report dealt with the Hazard Identification as part of a Risk Assessment, which meant an inventory of the moulds and toxic metabolites mainly associated with afore mentioned substrates.

This report deals with the Hazard Characterisation, an inventory of the (adverse) health effects that can occur in man and animals. Also, bioassays, in use to identify (adverse) effects on biological systems, have been described.

The total number of known toxic metabolites of moulds (to be called mycotoxins hereafter) is enormous and still growing; the number of mycotoxins known to cause adverse health effects in man and animals is much smaller. Some of these mycotoxins have been investigated in great detail, like aflatoxins; the knowledge of other mycotoxins is more superficial.

Aflatoxins have been studied more extensive because of their, first suspected and later on proven, carcinogenicity in mammals.

From other compounds carcinogenicity has been proven in animals while they are suspected to be carcinogenic to man. The evidence for carcinogenicity in man is still circumstantial. This is the case for fumonisin B_1, a *Fusarium* metabolite that has been related to human oesophageal cancer in certain parts of the world (Transkei and China). Fumonisin B_1 is also related to animal diseases, namely equine leukoencephalomalacia (ELEM) and porcine pulmonary edema (PPE). Involvement of fumonisin B_1 in these diseases has been proven.

Ochratoxin A, a mycotoxin of *Aspergillus* and *Penicillium* species, is related to a human disease, Balkan Endemic Nephritis.

These are examples of mycotoxins and diseases that are related through circumstantial evidence or are under strong suspicion of being related.

There are numerous investigations linking mycotoxins to different kinds of disorders in animals, as can be read in this report. The list of articles and different researches discussed here is by far not all that can be found on investigations with mycotoxins. It has never been the intention to give a full

overview of all the literature and all the investigations related to mycotoxins. Main goals for this report were to give a review of adverse health effects that can occur and to gather insight on the involvement of mycotoxins in human disease.

Numerous disorders have been found in experimental animals but no or scarce data on disorders in man are available. Investigations to gather such data are difficult to carry out and we lack the test systems to investigate possible adverse health effects in man. Therefore we lack the knowledge on adverse health effects of different mycotoxins in man.

Some test systems have been described, as can be read in the chapter on bioassays.

Some of these described systems are able to designate a specific effect to a compound, as for example being mutagenic. The number of such effect specific test systems is, however, limited.

Another problem is how to relate certain effects with the amount of mycotoxin necessary to achieve a certain effect. In some cases in this report amounts of mycotoxins to achieve a certain effect have been mentioned. How these amounts relate to the amount of normal intake, an exposure assessment, was not an objective of this report and as a consequence has not been given here.

Recently a risk assessment study on fumonisin B_1 exposure through the consumption of maize in The Netherlands has been published [192]. This thesis, however, is one of the very few complete studies concerning this problem. Such studies should also be undertaken, for example, for trichothecenes, a group of *Fusarium* mycotoxins able to cause a range of adverse health effects, like immunotoxic effects, in experimental animals. No such data on human exposure and human health risk are available, however.

Another field of research is the toxicity of combinations of mycotoxins. Some organisms are able to produce more than one mycotoxin, not only in laboratory conditions but probably too naturally. It has been found that naturally contaminated cereals are more toxic (often by a factor of two) than one would expect from the presence of known pure mycotoxins. This is due, presumably, to the presence of, and interaction with other identified, or as yet unidentified mycotoxins or metabolites [80].

In conclusion can be said that mycotoxins present in cereals and cereal products can cause a great number of adverse health effects in animals. Also in man direct adverse health effects due to exposure to contaminated cereals and cereal products have been reported. As some mycotoxins like ochratoxin A and fumonisin B_1 may accumulate in animal tissue[167 193], indirect exposure due to the consumption of meat from animals that have been fed cereals and cereal products contaminated with such mycotoxins also poses a health risk to man. Yet, there is still a great lack of knowledge

about the impact on man of exposure to mycotoxins. To come to a risk assessment on the exposure to mycotoxins in cereals and grain products, as this project aims to do, more investigations on the biological effects of mycotoxins on man have to be carried out. Moreover, more specific test sytems to investigate in vitro biological effects of mycotoxins will have to be designed.

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Appendix 1 Mailing list

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