ELSEVIER

Environmental Toxicology and Pharmacology 00 (2002) 1–12

ENVIRONMENTAL TOXICOLOGY AND PHARMACOLOGY ETAP

www.elsevier.com/locate/etap

Cytotoxicity assays for mycotoxins produced by *Fusarium* strains: a review

Arno C. Gutleb [a,*], Ellen Morrison [a], Albertinka J. Murk [b]

[a] *National Veterinary Institute, PO Box 8156 Dep., N-0033 Oslo, Norway*
[b] *Toxicology Section, Wageningen University, Tuinlaan 5, NL-6703 HE Wageningen, The Netherlands*

Abstract

Mycotoxins are naturally occurring toxic secondary metabolites of fungi that may be present in food and feed. Several of these mycotoxins have been associated with human and animal diseases. *Fusarium* species, found worldwide in cereals and other food types for human and animal consumption, are the most important toxigenic fungi in northern temperate regions. The overall economical loss and the detrimental health effects in humans and animals of mycotoxin contamination are enormous and therefore, rapid screening methods will form an important tool in the protection of humans and animals as well as to minimize economical losses by early detection. An overview of methods for the determination of cytotoxicity and the application of such bioassays to screen solid fungal cultures, cereals, respectively, food/feedstuffs for the presence and toxic potential of *Fusarium* mycotoxins is presented. Various cell lines including different endpoints of toxicity using vertebrate cells and the predictive value of the in vitro assays are reviewed. Bioassays are compared with existing chemical analytical methods and the possibilities and limitations of such systems are discussed. The review is based on 157 references. © 2002 Published by Elsevier Science B.V.

Keywords: *Fusarium*; Bioassay; Mycotoxins; In vitro

1. Introduction to mycotoxins

Mycotoxins comprise a group of chemically diverse compounds originating from secondary metabolism of moulds (filamentous fungi) and are mainly produced by five genera: *Aspergillus*, *Penicillium*, *Fusarium*, *Alternaria* and *Claviceps* (Steyn, 1995). The pattern and amounts of mycotoxins produced by a certain strain varies from year-to-year and depends on a plethora of factors including the species of crop as well as climatic and storage conditions (Marasas, 1991; Placinta et al., 1999). Many mycotoxins are stable under normal food processing conditions and can therefore, be present not only in food and feed but also in processed products (Hopmans and Murphy, 1993; Lauren and Smith, 2001).

The economical loss and health effects in humans and animals of mycotoxin contamination are enormous and therefore, rapid screening methods will form an important tool in the protection of humans and animals as well as to minimize economical losses by early detection. Mycotoxicoses may result from contaminated food, both locally grown and imported and current trade patterns and globalisation will increase the risk of such incidences if no precautionary measures such as strict control of products will be taken.

Fusarium species, found worldwide in cereals and other food types for human and animal consumption (Placinta et al., 1999), are the most important toxigenic fungi in northern temperate regions (Chelkowski, 1989; Nijs et al., 1996, 1997; Langseth et al., 1997, 1999; Parry et al., 1995; Abramson et al., 2001) and form a great economical problem for the agricultural sector (Charmley et al., 1994). About 20% of the crops grown in the European Union for foods and animal feeds contain measureable amounts of mycotoxins (Smith et al., 1994). *Fusarium* are considered to be field fungi, since they are primarily plant pathogens, but some species are exceptional and can grow in stored grain especially when water is freely available (a_w close to 0.99) and temperature low (Smith et al., 1994; Langseth et al., 1997). The occurrence of 61 different *Fusarium* species is described and at least 35 have been reported to produce mycotoxins, of which trichothecenes, fumonisins, zear-

* Coressponding author. Tel.: +47-23-216-224; fax: +47-22-597-453.
E-mail address: arno.gutleb@vetinst.no (A.C. Gutleb).

1382-6689/02/$ - see front matter © 2002 Published by Elsevier Science B.V.
PII: S1382-6689(02)00020-0

alenone and moniliformin were most frequently found (Placinta et al., 1999). Although most *Fusarium* mycotoxines have been detected worldwide there are regional differences in the relative importance, i.e. Fumonisins posing a larger problem in the Southern hemisphere than in the Northern (Kuiper-Goodman, 1995), or type B trichothecenes being more important in Europe and North America than type A trichothecenes (Pettersson, 1995; Fink-Gremmels, 1999).

The possible health risks of eposure to mycotoxins demand regular testing of food and feed. The interest for developing cellular systems for toxicity testing has increased in the last years as they are usually less expensive, are more quantitative and rapid, and avoid ethical considerations inherent to any in vivo experiment. Chemical analysis of large sample numbers is both expensive and time- and labour consuming and to assay chemically for all known mycotoxins is almost impossible. As with other functional in vitro tests it is important to determine the response level above which adverse effects may be expected. This is necessary to be able to use in vitro tests as single tests but still needs additional research. Cellular systems have their strength as a screening tool for biological and toxicological activity and to identify samples for which a chemical analysis is justified.

In this review the main groups of *Fusarium* mycotoxins, and their toxicological relevance are briefly presented. The available literature concerning bioassays based on vertebrate cells to screen and determine the presence and toxicological significance of *Fusarium* mycotoxins is discussed thereafter.

2. Important *Fusarium* mycotoxins

Trichothecenes constitute the largest group of *Fusarium* mycotoxins. Altogether 150 trichothecenes and trichothecene derivatives have been isolated and characterized. They are tricyclic sesquiterpenes characterized by the presence of a double bond at C-9,10 and an epoxy-ring at C-12,13 and consequently are classed as 12,13-epoxy-trichothecenes. The distinctive chemical features of these molecules, has led to the designation of four subclasses. Type A has a functional group other than a ketone at position C-8, whereas Type B has a ketone at C-8. Type C has a second epoxy group at C-7,8 or C-9,10. Type D contains a macrocyclic ring between C-4 and C-5 with two ester linkages. Only a few of the known trichothecenes, (all of type A or B) seem to be of importance with respect to the presence in crops, whilst for the majority of trichothecenes that have been demonstrated under laboratory conditions there is little evidence for their occurence under field conditions (Smith et al., 1994).

Mass toxicoses in humans with trichothecenes have been observed in the past (Beardall and Miller, 1994; Peraica et al., 1999). Type A trichothecenes such as T-2 toxin, HT-2 toxin, neosolaniol and diacetoxyscirpenol (DAS) are more acutely toxic whereas type B trichothecenes such as deoxynivalenol (vomitoxin) (DON) and nivalenol (NIV) are more less so, and are therefore, implicated in more chronic toxicoses (Prelusky et al., 1994; Rotter et al., 1996). Common symptoms are lesions of the mucosa in the gastrointestinal tract resulting in extensive haemorrhage, a general inflammatory response (Fink-Gremmels, 1999), and haematological toxicities (Joffe, 1974, 1978). The typical symptom for acute, high dosage trichothecene ingestion is vomiting and feed refusal (Williams et al., 1989).

Trichothecenes, including the most toxic T-2 toxin, inhibit both protein synthesis and mitochondrial function in vitro (Oldham et al., 1980; Holt and DeLoach, 1988) and in vivo (Rosenstein and Lafarge-Frayssinet, 1983). Both Type A and Type B trichothecenes showed in vitro and in vivo immunsuppressing effects even at concentrations lower than what is found in cereals causing epidemics, and may thereby influence incidence of secondary diseases (Parent-Massin et al., 1994; Rafai, et al., 1995; Johannisson et al., 1999; Berek et al., 2001). T-2 toxin induces apoptosis (Ihara et al., 1997; Shinozuka et al., 1997) and alters multiple cell membrane functions at concentrations from 0.9 to 9 nM (Bunner and Morris, 1988). This effect can be counteracted by co-incubation with antioxidants (Shokri et al., 2000). DAS exposure caused suppression in macrophage phagocytic function (Qureshi et al., 1998).

DON is not as toxic as other trichothecenes but it is a very stable compound during both storing and processing of food, and does not degrade at high temperatures (Scott, 1991). DON had a direct effect on morphology and function of cultured adenocarcinoma cells thereby resembling the observed in vivo effects (Kasuga et al., 1998). Furthermore DON inhibited human and rat granulomonocytic progenitors ($EC_{50} < 40$ nM) (Lautraite et al., 1997). NIV increased cytochrome P-450 and glutathione S-transferase activities in rats thereby increasing the formation of aflatoxin B_1 adduction to DNA (Yabe et al., 1993).

Fumonisins are an increasingly important group of toxins (Gelderblom et al., 1988; Norred, 1993; Norred and Voss, 1994; Dutton, 1996) as they have been postulated as the causative agent for several endemic diseases both in humans and domestic animals (Chu and Li, 1994; Marasas et al., 1988; Sydenham et al., 1990).

The basic chemical structure is a diester of propane-1,2,3-tricarboxylic acid and a pentahydroxyicosane containing a primary amino group. Fumonisins have a marked similarity to the brain lipid sphingosine. Members of the A series are acetylated on the amino group, are only produced in small quantitities, and have low or

no biological activity (Gelderblom et al., 1988; Abbas et al., 1993). Members of the Fumonisin C-series are structurally identical to the B series but lack the C-1 terminal methyl group (Branham and Plattner, 1993; Seo et al., 1996). Fumonisin P_{1-3} are the last group described and are characterized by a 3-hydroxypyridinium functional group on the C-2 amine group (Musser et al., 1996). The fumonisin B series, including FB_1, FB_2, FB_3, and FB_4 are the most abundant in contaminated food and are generally the most toxic to experimental animals (Abbas et al., 1998).

The presence of fumonisins, mostly found together with moniliformin, has important health implications and the known alterations described in vertebrates comprise equine leukoencephalomalacia (Marasas et al., 1988), porcine pulmonary oedema (Harrison et al., 1990), immunodepressive effects in turkey poults (Li et al., 2000), and vitamin A reduction in chicks (Hall et al., 1995). Fumonisin B_1 has been linked to human oesophageal cancer in several countries (Chu and Li, 1994; Sydenham et al., 1990). Furthermore fumonisins are known to be hepatocarcinogenic (Gelderblom et al., 1991). They are strong inhibitors of protein synthesis (Norred et al., 1990), ceramide synthetase (Wang et al., 1991) and are able to induce chromosomal aberrations in rat hepatocytes (Knasmüller et al., 1997).

Zearalenone is produced by *Fusarium graminearum*, *Fusarium culmorum*, *Fusarium equiseti*, *Fusarium poae* and some other *Fusarium* species and is commonly found in corn and corn products. Zearalenone is a macrocyclic lactone with high binding affinity to oestrogen receptors (Kuiper et al., 1997) and only low acute toxicity. In mammals it causes an oestrogen-like syndrome in both sexes (infertility, vulval oedema, vaginal prolapse and mammary hypertrophy in females and feminisation in males) at concentrations in feeds higher than 20 ng/g with pigs being the most sensitive species (Blaney et al., 1984; Dacasto et al., 1995; Smith et al., 1994). Concentrations exceeding 50 ng/g in the diet of pigs will lead to constant oestrus, a pseudopregnancy syndrome and complete infertility (Diekman and Green, 1992).

Moniliformin (1-hydroxycyclobut-1-ene-3,4-dione) is produced by several *Fusarium* species (Table 1). Moniliformin is suspected to be associated with an endemic disease in China called Keshan disease, characterized as cardiomyopathy (Zhu et al., 1982; Chen et al., 1990). In poultry both acute and chronic effects have been described with progressive muscular weakness, respiratory distress, cyanosis, coma, and death being the most prominent symptoms (Kriek et al., 1977). Acute mortality and gross lesions, including ascites, hydropericardium, and myocardial pallor have been observed in broilers, turkeys and ducklings (Engelhardt et al., 1989; Leodux et al., 1995). Moniliformin was shown to inhibit the pyruvate dehydrogenase complex (Thiel, 1978; Burka et al., 1982; Gathercole et al., 1986), and gluconeogenesis in cultured primary chicken hepatocytes (Wu and Vesonder, 1997). Moniliformin induced chromosomal aberrations in rat hepatocytes (Knasmüller et al., 1997).

3. The application of cytotoxicity studies in mycotoxin analysis

A number of chemical methods for the determination of most of the known *Fusarium* mycotoxins have been developed (Langseth and Rundberget, 1998; Krska and Josephs, 2001a; Krska et al., 2001b), although a lack of simple and reliable screening methods at low costs for both type A- and type-B trichothecenes has been stated recently (Krska et al., 2001b). Additive, synergistic and antagonistic effects have been reported for mixtures of mycotoxins both in vivo and in vitro (Huff and Doerr, 1981; Javed et al., 1993; Huff et al., 1988; Kubena et al., 1995, 1997; Groten et al., 1998; Boeira et al., 2000; Gelderblom et al., 2002). The combinatory effects of already known compounds and eventually present uncharacterised metabolites cannot be estimated solely from chemical analyses and are by far not fully understood yet. To study both the toxicity and mode of actions of mycotoxins is still a challenge and scientifically sound in vitro systems are a need to refine, replace and reduce the use of animals in biomedical research.

In addition to vertebrate cell lines (this review) many different organisms, such as single cell organisms, plants, and various animals (protozoa, dinoflagellate, crustaceans, fish, nematodes, insects, bird eggs, birds, mammals) have been used in the past (Buckle and Sanders, 1990; Eppley, 1974; Forgacs et al., 1954; Yates and Porter, 1982; Prelusky et al., 1987; Vesonder et al., 1990; Vesonder et al., 1992; Vesonder et al., 2000; Panigrahi, 1993). Several bioassays have been based on yeast strains (Yates, 1986; Madhyastha et al., 1994; Binder, 1999; Engler et al., 1999).

3.1. Type of cells

Utilizing mammalian cell lines for assays of possible toxicity are established methods (Grove and Mortimer, 1969; Saito et al., 1971; Lompe and Milczewski, 1979; Joffe, 1986; Robb and Norval, 1985; Robbana-Barnat et al., 1989). Through the years, many different cell lines and primary cells have been used in research related to *Fusarium* mycotoxins (Tables 1–4). Application of more than one cell line of different origin for screening purposes has been recommended, as different sensitivities of different cell lines were observed already more than two decades ago (Lompe and Milczewski, 1979; Robb and Norval, 1983; Babich and Borenfreund, 1991). For example only eight out of 26 different cell

Table 1
EC_{50} concentrations (μM) for type A trichothecenes, T-2 toxin (T-2), HT-2 toxin (HT-2), 4,15-diacetoxyscirpenol (DAS), neosolaniol (NEO)

Cell line	Species	Cell type	T-2	HT-2	DAS	NEO	Parameters	References
Chang	Human	Liver epithelial	0.02[b]				Visual	Robb and Norval, 1983
3T3	Mouse	Fibroblast	0.2	176.0	20.4	26.1	Visual	Abbas et al., 1984a,b
PBM	Bovine	Mononuclear	0.4				[^{3}H]-thimidine	Charoenpornsook et al., 1998
HGF[a]	Human	Gingival fibroblast	0.5				Neutral red	Shokri et al., 2000
KA31T[a]	Mouse	Fibroblast	0.6				Cell counts	Shier et al., 1991
MIN-GL1[a]	Human	Lymphoid	0.7	5.9	2.7	130.0	MTT	Visconti et al., 1991
BHK-21	Hamster	Fibroblasts	0.9	10.7	27.3		[1-^{14}C]-leucine	Scossa-Romano et al., 1987
GM498[a]	Human	Skin fibroblast	> 1.1				Cell counts	Shier et al., 1991
K-562[a]	Human	Erythroleukemia	2.1	47.1	2.7	392.0	MTT	Visconti et al., 1991
MDBK[a]	Bovine	Kidney	2.2				[^{3}H]-leucin	Holt and DeLoach, 1988
Primary	Mouse	Splenic lymphocyte	2.4	7.3	16.1		[^{3}H]-thimidine	Porcher et al., 1987
BHK-21	Hamster	Fibroblast	3.4	9.2	6.6	86.3	MTS	Rotter et al., 1993
CHO[a]	Hamster	Ovary	3.7				[^{3}H]-leucin	Thompson and Wannemacher, 1984
MDBK[a]	Bovine	Kidney	4.7				MTT	Holt et al., 1988
SK-Mel/27[a]	Human	melanoma	6.0				Neutral red	Babich and Borenfreund, 1991
GM3349	Human	Fibroblast	6.4				Visual	Abbas et al., 1984a,b
Primary	Human	Fibroblast	8.6				[^{3}H]-thimidine	Oldham et al., 1980
3T3	Mouse	Fibroblast	10.0	30.0			BrdU	Widestrand et al., 1999
SW742[a]	Human	Adenocarcinoma	11.8				Neutral red	Shokri et al., 2000
NCTC clone 929[a]	Mouse	Connective tissue	16.4				[^{3}H]-leucin	Thompson and Wannemacher, 1984
CHO-K1	Hamster	Ovary	21.0				[^{3}H]-leucin	Holt et al., 1987
CHO[a]	Hamster	Ovary	22.2				[^{3}H]-leucin	Holt and DeLoach, 1988
CHO	Hamster	Ovary	37.1	63.6		272.0	[^{3}H]-leucin	Thompson and Wannemacher, 1986
V79[a]	Hamster	Kidney	56.0				MTT	Holt et al., 1988
L-6	Rat	Myoblast	75.0				Rubidium uptake	Bunner and Morris, 1988
NK[a]	Human	Primary kidney	154.6				Neutral red	Babich and Borenfreund, 1991

[a] More than two cell lines were tested; only the cell lines with the lowest, respectively, the highest EC_{50} are included in Table 1.
[b] Lowest concentration that gave an significant effect, no EC_{50} data are given.

lines were sensitive to Fumonisin B_1 and B_2 with EC_{50} concentrations ranging from 2.8 to 69.3 μM (Shier et al., 1991). On the other hand, T-2 toxin often used as a positive control due to its high cytotoxicity to most cultured cell lines (Abbas et al., 1984a,b), exerted cytotoxicity in all of the 26 cell lines. Nevertheless, the EC_{50} concentrations (0.6–10,7 nM) differed by a factor of 18 for T-2 toxin (Shier et al., 1991). EC_{50} concentrations for T-2 toxin ranging from 4.7 to 56 μM were found in a battery of ten cell lines (Holt et al., 1988). In the same experimental series primary lymphocytes were more sensitive to T-2 toxin than most of the tested cell lines with mouse lymphocytes being less sensitive (6.6 μM) than rat lymphocytes (2,7 μM) (Holt et al., 1988). Similar differences in susceptibility were observed for cytotoxic effects of T-2 toxin in MDBK (bovine kidney) and Chinese hamster ovary (CHO) cells, where CHO cells were ten times less sensitive than MDBK (Holt and DeLoach, 1988). Of eight tested cells primary human kidney cells were more than 25 times less sensitive to T-2 toxin than SK-Mel/27, a melanoma cell line, in which an EC_{50} of 6 μM was found (Babich and Borenfreund, 1991).

Table 2
EC_{50} concentrations (μM) for type B trichothecenes, 4-deoxynivalenol (DON), nivalenol (NIV)

Cell line	Species	Cell type	DON	NIV	Parameters	References
K562	Human	Erythroleukemia	1.35[a]		MTT	Reubel et al., 1987
PBM	Bovine	Mononuclear	135		[^{3}H]-thimidine	Charoenpornsook et al., 1998
BHK-21	Hamster	Fibroblast	378	269	MTS	Rotter et al., 1993
Primary	Mouse	splenic lymphocyte	388		[^{3}H]-thimidine	Porcher et al., 1987
BHK-21	Hamster	Fibroblast	705		[1-^{14}C]-leucine	Scossa-Romano et al., 1987
3T3	Mouse	Fibroblast	900	1100	BrdU	Widestrand et al., 1999
K-562[a]	Human	Erythroleukemia	1010	960	MTT	Visconti et al., 1991
MIN-GL1[a]	Human	Lymphoid	1350	640	MTT	Visconti et al., 1991
VERO	Monkey	Kidney	1500	8100	[^{3}H]-leucin	Thompson and Wannemacher, 1986
3T3	Mouse	Fibroblast	1600	320	Visual	Abbas et al., 1984a,b

[a] More than two cell lines were tested; only the cell lines with the lowest, respectively, the highest EC_{50} are included in Table 1.

Table 3
EC_{50} concentrations (μM) for fumonisin B_1–B_3 and C_{1-4}

Cell line	Species	Cell type	FB_1	FB_2	FB_3	FC_{1-4}min-max	Parameters	References
Primary	Rat	Hepatocyte	0.1				Sphingolipid biosynthesis	Norred et al., 1992
MDCK	Dog	Kidney	12	20	4.7	38.3–316	Visual	Abbas et al., 1993, 1998
LLC-PK1	Pig	Kidney	35				Sphingolipid biosynthesis	Norred et al., 1992
H4TG	Rat	Hepatoma	58	1.7	56.7	14.0–49.4	Visual	Abbas et al., 1993, 1998
NIH3T3	Mouse	Fibroblast	> 200	–[a]	–[a]	> 200	Visual	Abbas et al., 1993, 1998
Primary	Chicken	Chondrocyte	> 200				MTT	Wu et al., 1995a,b
Primary	Chicken	Splenocyte	> 200				MTT	Wu et al., 1995a,b
Primary	Turkey	Lymphocyte	1940	550			MTT	Dombrink-Kurtzman et al., 1994
MDCK	Rat	Hepatoma	3460	2830			cell counts	Shier et al., 1991
BHK-21	Hamster	Fibroblast	Ne				neutral red	Vesonder et al., 1993
MM	Mouse	Fibroblast	Ne				neutral red	Vesonder et al., 1993
H4IIE	Rat	Hepatoma	Ne				neutral red	Vesonder et al., 1993
H4IIE	Rat	Hepatoma	Ne				neutral red	Vesonder et al., 1993
MDCK	Dog	Kidney	Ne				neutral red	Vesonder et al., 1993
CHO	Hamster	Ovary	Ne				neutral red	Vesonder et al., 1993

Ne, no effect.
[a] No detectable cytotoxicity at 70 μM fumonisin B_2 and B_3.

Remarkable differences between studies of the EC_{50} concentrations for identical cell lines were reported. With CHO cells the EC_{50} concentrations of T-2 toxin differed by a factor of six (Thompson and Wannemacher, 1986; Holt and DeLoach, 1988) and in VERO cells, a monkey kidney line, by a factor of 3.3 (Thompson and Wannemacher, 1986; Shier et al., 1991). This was independent of the chosen parameter of cytotoxicity, though [^{3}H]-leucin was used in both studies to measure inhibition of protein synthesis in the first example.

4. Type of endpoint

4.1. General cytotoxicity

Visual inspection or staining of cells followed by cell counting was used in several studies to determine toxic effects of mycotoxins (Robb and Norval, 1983; Abbas et al., 1984a,b, 1993, 1998; Vesonder et al., 1993; Shier et al., 1991, 2001). The results of methods based upon visual inspection are somewhat questionable due to the subjectivity of evaluation and method of assessing viability. This is emphasized by the fact that results of

Table 4
EC_{50} concentrations (μM) for zearalenone (ZON) and moniliformin (MON)

Cell line	Species	Cell type	ZON	MON	Parameters	References
MCF-7	Human	Adenocarcinoma	0.0002		DNA	Welshons et al., 1990
MCF-7	Human	Adenocarcinoma	0.0006		Exoprotein	Mayr et al., 1992
MCF-7	Human	Adenocarcinoma	0.001		Cell counting	Shier et al., 2001
Primary	Human	Lymphocyte	15	23	MTT	Visconti et al., 1991
HEp-II	Human	Epithelial	312[a]	Nd[b]	Visual	Robb and Norval, 1983
Chang	Human	Liver epithelial	312[a]	Nd[b]	Visual	Robb and Norval, 1983
K-562	Human	Erythroleukemia	312	> 1000	MTT	Visconti et al., 1991
MIN-GL1	Human	Lymphoid	> 312	> 1000	MTT	Visconti et al., 1991
3T3	Mouse	Fibroblast	6200		Visual	Abbas et al., 1993, 1984a,b
Primary	Mouse	Splenic lymphocyte	10000		[^{3}H]-thimidine	Porcher et al., 1987
Primary	Chicken	Skeletal myocyte		42	MTT	Wu et al., 1995a,b
Primary	Chicken	Cardiac myocyte		95	MTT	Wu et al., 1995a,b
BHK-21	Hamster	Fibroblast		nd[c]	Neutral red	Vesonder et al., 1993
H4IIE	Rat	Hepatoma		100	Neutral red	Vesonder et al., 1993
MDCK	Dog	Kidney		100	Neutral red	Vesonder et al., 1993
MM	Mouse	Fibroblast		255	Neutral red	Vesonder et al., 1993
SK	Pig	Kidney		> 500 000	MTT	Hanelt et al., 1994
Hep-II	Human	Epithelial		nd	Fluorescence flow cytometry	Robb et al., 1990

nd, No detectable cytotoxicity.
[a] Lowest levels with detectable cytotoxicity.
[b] No detectable cytotoxicity at 10 mM.
[c] No detectable cytotoxicity at 768 μM.

visual inspection in T-2 toxin treated 3T3, a mouse fibroblast cell line, were almost 50 times more sensitive than results obtained with a DNA synthesis assay (incorporation of 5-bromo-2′-deoxyuridine- BrdU) test (Abbas et al., 1984a,b; Widestrand et al., 1999).

The neutral red (NR) (3-amino-7-dimethylamino-2-methyl-phenazine hydrochloride) cytotoxicity assay is based on the incorporation of the supravital dye neutral red, into the lysosomes of viable cells. Compounds that injure the plasma or lysosomal membrane decrease uptake and subsequent retention of the dye. NR was successfully used to determine the cytotoxic effects of trichothecene toxins (Babich and Borenfreund, 1991; Vesonder et al., 1993; Shokri et al., 2000).

Several studies report the use of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) cleavage test, which monitors a reduction of yellow tetrazolium salt by mitochondrial dehydrogenase enzymes of metabolically active/viable cells to purple formazan crystals. The formation of formazan can be analysed automatically after solubilization and was used in several studies (Reubel et al., 1987; Holt et al., 1988; Visconti et al., 1991; Dombrink-Kurtzman et al., 1994; Hanelt et al., 1994; Wu et al., 1995a,b). The structurally related compound MTS (5-(3-carboxymethoxyphenyl)-2-(4,5-dimethylthiazol-2-yl)-3-sulfophenyl) tetrazolium) has the advantage of the formation of a water-soluble formazan and has also been applied in mycotoxin related studies (Rotter et al., 1993).

4.2. Specific endpoints

All above-mentioned methods are unspecific, as they merely measure general cytotoxicity (dead/viable) or reduced cell multiplication. More specific endpoints in a battery of cell types should therefore, be applied both to achieve lower detection limits and to increase the insight in quantitative relationships between dose and response. Such an approach should increase the predictive value of in vitro assays with regard to the toxic properties of the mycotoxins. In a systematic approach using hepatocytes and lymphocytes as test systems the cytotoxicity of nine mycotoxins correlated with data from literature on in vivo toxicity. Aflatoxin B_1 for example acted preferentially on hepatocytes. T-2 toxin, a well known in vivo immunsuppressor showed lymphotrophic effects, whereas citrinin, a mycotoxin with known renal toxicity had no effects on hepatocytes and lymphocytes (Robbana-Barnat et al., 1989). It has to be stated clearly at this point that usually no rationale for the choice of cell types is given in the literature and the actual choice may be more a matter of chance than that it is based on available knowledge about the mode of action of a certain mycotoxin.

Type A trichothecenes (Tables 1 and 2) were shown to significantly alter multiple cell membrane functions, such as calcium efflux, ^{86}rubidium uptake, or residual cellular lactate dehydrogenase (LDH) in L-6 myoblasts at concentrations as low as 9 nM, although the EC_{50} of ^{86}rubidium uptake, the most sensitive parameter, was much higher (75 μM) (Bunner and Morris, 1988). The effects were observed as soon as 10 min after exposure and may provide a rapid and sensitive endpoint. Inhibition of protein synthesis measured by [^{3}H]-leucin incorporation in bovine peripheral blood mononuclear cells was more sensitive ($EC_{50} = 0.4$ μM) than general cytotoxicity assays including MTT or LDH measurement (Charoenpornsook et al., 1998). Cell lines were less sensitive than these bovine peripheral blood mononuclear cells with EC_{50} concentrations for T-2 toxin ranging from 16.4 up to 37.1 μM T-2 toxin (Thompson and Wannemacher, 1984). EC_{50} values for T-2 toxin and HT-2 toxin were found to be similar in primary mouse lymphocytes (2.4 and 7.3 μM, respectively; Porcher et al., 1987). Lymphocytes are more sensitive to T-2 toxin than other cell types such as kidney cells (Table 1) and this corresponds well with data from in vivo experiments that showed that trichothecenes act as immunosuppressive agents (Holladay et al., 1993; Javed et al., 1995; Neiger et al., 1994). Inhibition of DNA and protein synthesis that is used as a sensitive endpoint in cell systems corresponds with the observed in vivo changes in serum chemistry of pigs (Harvey et al., 1990).

Lymphoid cells and fibroblasts were the most sensitive cell types to DON (Reubel et al., 1987; Charoenpornsook et al., 1998; Rotter et al., 1993). DON induced significant changes in the human colonic adenocarcinoma cell lines Caco-2 and T84 with transepithelial electrical resistance, lucifer yellow permeability, alkaline phosphatase activity and sucrase-isomaltase activity as endpoints (Kasuga et al., 1998). DON is known to effect both the gastrointestinal tract and the immunsystem (Rotter et al., 1996).

In general other trichothecenes were much less cytotoxic than T-2 toxin, independent of the applied endpoint and the cell type (Abbas et al., 1984a,b; Visconti et al., 1991; Scossa-Romano et al., 1987; Porcher et al., 1987; Rotter et al., 1993; Thompson and Wannemacher, 1986; Reubel et al., 1987; Widestrand et al., 1999; Charoenpornsook et al., 1998).

Fumonisins exert their toxic effects through the disruption of sphingolipid metabolism by inhibiting ceramide synthetase both in vitro (Riley et al., 1996; Yoo et al., 1996) and in vivo (Bucci et al., 1998; Solfrizzo et al., 2001). This mechanism resulted in accumulation of free sphingoid bases and depletion of complex sphingolipids, such as sphingomyelin and glycosphingolipids in LLC-PK1, a pig kidney cell line with an EC_{50} concentration of 35 μM (Norred et al., 1992). The ratio of sphinganine:sphingosine has been successfully applied as a biomarker for fumonisin exposure and contamination (Riley et al., 1993, 1994). The structu-

rally related *Alternaria alternata* toxin TA inhibited ceramid synthetase as well (Norred et al., 1996). Other mycotoxins that are structurally different from fumonisins do not alter the ratio even at concentrations causing general cytotoxicity (Norred et al., 1997). Attempts to establish a bioassay with BHK-21 cells using other endpoints, such as RNA, DNA or protein synthesis failed, due to lack of consistent response (Abeywickrama et al., 1998). The highest sensitivity for fumonisins was obtained using precision-cut rat liver slice, though not representing classical cell culture (Norred et al., 1996, 1997).

Zearalenone is known for its high binding affinity to oestrogen receptors (Kuiper et al., 1997). Zearalenone induced oestrogen-responsive proliferation of MCF-7 cells (human breast cancer cells) at levels of 200 pM (Welshons et al., 1990). MCF-7 cells are extremely sensitive, superior to the in vivo uterotrophic assay with mice and rats for the determination of zearalenone both for purified compounds and for biological material (Mayr, 1990). Recently, zearalenone and especially the metabolite α-zearalenone, were found to be as strong estrogens as 17-α-ethynil estradiol. Results were obtained in three different bioassays, using activation of reporter gene in recombinant yeast, vitellogenin gene induction of rainbow trout hepatocyte cultures and alkaline phosphatase gene induction in human endometrial Ishikawa cell line (Le Guevel and Pakdel, 2001). Contrary to these sensitive cells, primary mouse lymphocytes were insensitive to zearalenone with EC_{50} concentrations as high as 10 mM and proteins synthesis was not altered (Porcher et al., 1987). The results obtained in vitro with estrogenic sensitive cell lines resemble the oestrogenic like effects of zearalenone in vivo (Dacasto et al., 1995; Yang et al., 1995; Mehmood et al., 2000).

Moniliformin has a unique mode of action by inhibiting the pyruvate dehydrogenase complex (Thiel, 1978; Burka et al., 1982; Gathercole et al., 1986) that results in vivo in myopathy (Kriek et al., 1977; Engelhardt et al., 1989; Leodux et al., 1995; Morris et al., 1999). Of six different primary chicken cell types myocytes and cardiomyocytes were most sensitive to moniliformin (Wu et al., 1995a,b). The toxicity to myocytes was further ascertained as the contractile force of muscle strips was significantly reduced, by decreasing the open probability of sarcoplasmatic reticular Ca^{2+} channels (Nagaraj et al., 1996). Creatine phosphokinase in L6 myoblasts was also used as an endpoint (Reams et al., 1996). All other experiments with moniliformin have been performed using cell viability or other general parameters as a measure for toxicity, with higher EC_{50} concentrations than for all other mycotoxins (Visconti et al., 1991; Robb and Norval, 1983; Abbas et al., 1984a,b; Wu et al., 1995a,b; Porcher et al., 1987; Hanelt et al., 1994).

5. The application of in vitro tests to matrix extracts

All the above experiments were performed using purified standards. For the relevance of any cytotoxicity test the ability to detect combinations of mycotoxins, respectively, co-occurring mycotoxins present either in fungal cultures or food/feed extracts is more important.

Fungal extracts of *Fusarium* strains were tested on human and bovine lymphocytes, and cytotoxicity was correlated to the chemically analysed T-2 toxin concentrations in the strains (Visconti et al., 1992a,b). Both organic and aqueous extracts of *F. moniliforme* cultures were cytotoxic to rat primary hepatocytes. This cytotoxicity could not be accounted to the chemically analysed concentrations of fumonisin B_1 and fusarin C, thereby showing the ability of cell systems to detect toxic compounds for which no standards for chemical analysis are available (Norred et al., 1991). Purified 4-deoxynivalenol (DON) was much less cytotoxic then DON extracted from culture material in conjunction with co-occurring mycotoxins (Miller et al., 1983; Rotter et al., 1993). Recently toxicity of *Fusarium equiseti* rice culture extracts in PK15 (porcine kidney) cells was attributed to specific combinations of mycotoxins using stepwise multiple regression analysis (Morrison et al., 2002).

Grain extracts from Norway were cytotoxic to VERO and PK cells, as determined with the MTT assay, and toxicity was significantly correlated with the amount of *F. avenaceum* but not with concentrations of DON. The results indicated the presence of an unknown cytotoxic principle in overwintered grain (Langseth et al., 1997). For T-2 toxin spiked and naturally contaminated corn, a good correlation was found between EC_{50} concentration on lymphocytes and T-2 toxin in corn, when analysed by gaschromatography (GC-FID) (Porcher et al., 1987). Maize extracts (14 of 24) were toxic to BHK cells, determined by [1-^{14}C]-leucin incorporation. The results of the bioassay were confirmed chemically in 11 samples, and no thrichothecenes were detected in the 10 samples that were negative in the bioassay (Scossa-Romano et al., 1987). Several unidentified compounds from *Fusarium* contaminated grain associated with problems in broiler chicks in Scotland were found to be cytotoxic in addition to the four chemically identified mycotoxins (Robb et al., 1982), thereby showing the additional value of a bioassay.

6. Conclusions and outlook

Most studies of trichothecene toxicity used either fibroblast or lymphoid cell lines, and these cell types were characterized by low EC_{50} values, whereas cells of other origin appear to be less sensitive (Tables 1 and 2). For fumonisins, kidney cell lines were the most sensitive

(Table 3). As expected, estrogenic responsive tumor cells were most sensitive to zearalenone (Table 4), and myocytes showed greatest sensitivity to moniliformin (Table 4). These observations correspond with common knowledge of the toxic mechanisms of the compounds discussed and match the results of the great number of in vivo experiments. Nevertheless, it has to be stated, that there are great differences between cell lines derived from the same tissue in sensitivity to all *Fusarium* toxins and EC_{50} values are high in most of the reported systems (Tables 1–4). More attention should therefore, be given to the identification of sensitive cell lines for a given mode of action and to comparison of these results with in vivo responses. With increased knowledge of toxic mechanisms, more specific and sensitive endpoints, such as enzymatic effects, should be evaluated in the future. Non-radioactive methods should be preferred to minimize health risks and waste disposal problems. Some mycotoxins induce apoptotic processes that should also be further evaluated as a possible endpoint of bioassays.

In the future attention should be given to the role of multiple mycotoxin exposure and possible effects of mixtures. More attention to other *Fusarium* mycotoxins, such as culmorin, fusarin C, fusarochromanone, chlamydosporol, the class of enniatines, butenolide, or wortmannin is necessary, as data on cytotoxicity and mode of actions are almost completely lacking.

In addition to potential subjective errors, all methods based on visual inspection are time and labour consuming, and therefore, inappropriate for large scale screening purposes. Spectrophotometrical analysis of the metabolic conversion of dyes can be used to determine general cytotoxicity and may provide a more objective, faster and cheaper method, but do not contribute to the understanding of the specific mode of action of mycotoxins. A battery of systems based on several cell lines with specific endpoints may therefore, represent a more useful screening tool and will increase our insight in relationships between dose and response, at least when sufficient chemical, biological and toxicological validation has been performed.

7. Uncited references

Lewis et al., 1999; Pitt, 2000; Senter et al., 1991; Abbas et al., 1989, 1994; Visconti and Solfrizzo, 1995.

Acknowledgements

We would like to acknowledge the great contribution of Wenche Langseth to the awareness and knowledge of risks of mycotoxins in food and feed in Norway. Unfortunately, an unexpected severe illness put a premature end to her fruitful scientific work. This manuscript is part of Norges forskningsråd (Norwegian Research Council) financed project nr. 141221/130. Aksel Bernhoft and Silvio Uhlig are thanked for their valuable comment. The authors would like to thank the editor for the invitation to submit this review.

References

Abbas, H.K., Mirocha, C.J., Shier, W.T. 1984a. Mycotoxins produced from fungi isolated from foodstuffs and soil: comparison of toxicity in fibroblasts and rat feeding tests. Appl. Environ. Microbiol. 48, 654–661.

Abbas, H.K., Shier, W.T., Mirocha, C.J. 1984b. Sensitivity of cultured human and mouse fibroblasts to trichothecenes. J. Assoc. Off. Anal. Chem. 67, 607–610.

Abbas, H.K., Mirocha, C.J., Gunther, R. 1989. Mycotoxins produced by toxic *Fusarium* isolates obtained from agricultural and non-agricultural areas (arctic) of Norway. Mycopathologia 105, 143–151.

Abbas, H.K., Gelderblom, W.C.A., Cawood, M.E., Shier, W.T. 1993. Biological activities of fumonisins, mycotoxins from *Fusarium moniliforme* in jimsonweed (*Datura stramonium* L.) and mammalian cell cultures. Toxicon 31, 345–353.

Abbas, H.K., Tanaka, T., Duke, S.O., Porter, J.K., Wray, E.M., Hodges, L., Sessions, A.E., Wang, E., Merill, A.H., Riley, R.T. 1994. Fumonisin- and AAL-toxin induced disruption of sphingolipid metabolism with accumulation of free sphingoid bases. Plant Physiol. 106, 1085–1093.

Abbas, H.K., Shier, W.T., Seo, J.A., Lee, Y.W., Musser, S.M. 1998. Phytotoxicity and cytotoxicity of the fumonisin C and P series of mycotoxins from *Fusarium* spp. fungi. Toxicon 36, 2033–2037.

Abeywickrama, K., Bean, G.A., Kennedy, K.A. 1998. Effects of *Fusarium moniliforme* culture extracts and fumonisin B1 on DNA, RNA and protein synthesis by baby hamster kidney cells. Mycopathologia 143, 59–63.

Abramson, D., Clear, R.M., Gaba, D., Smith, D.M., Patrick, S.K., Saydak, D. 2001. Trichothecene and moniliformin production by *Fusarium* species from Western Canadian wheat. J. Food Protein 64, 1220–1225.

Babich, H., Borenfreund, E. 1991. Cytotoxicity of T-2 toxin and its metabolites determined with the neutral red cell viability assay. Appl. Environ. Microbiol. 57, 2101–2103.

Beardall, J.M., Miller, J.D. 1994. Diseases in humans with mycotoxins as possible causes. In: Miller, J.D., Trenholm, H.L. (Eds.), Mycotoxins in Grain. Eagan, St. Paul, MN, pp. 487–540.

Berek, L., Petri, I.B., Mesterházy, Á., Terén, J., Molnár, J. 2001. Effects of mycotoxins on human immune functions in vitro. Toxicol. In Vitro 15, 25–30.

Binder, J. 1999. A yeast bioassay for trichothecenes. Nat.Toxins 7, 401–406.

Blaney, B.J., Bloomfield, R.C., Moore, C.J. 1984. Zearalenone intoxication of pigs. Aust. Vet. J. 61, 24–27.

Boeira, L.S., Bryce, J.H., Stewart, G.G., Flannigan, B. 2000. The effect of combinations of *Fusarium mycotoxins* (deoxynivalenol, zearalenone and fumonisin B1) on growth of brewing yeasts. J. Appl. Microbiol. 88, 388–403.

Branham, B.E., Plattner, R.D. 1993. Isolation and characterization of a new fumonisin from liquid cultures of *Fusarium moniliforme*. J. Nat. Prod. 56, 1630–1633.

Bucci, T.J., Howard, P.C., Tolleson, W.H., Laborde, J.B., Hansen, D.K. 1998. Renal effects of fumonisin mycotoxins in animals. Toxicol. Pathol. 26, 160–164.

Buckle, A.E., Sanders, M.F. 1990. An appraisal of bioassay methods for the detection of mycotoxins—a review. Lett. Appl. Microbiol. 10, 155–160.

Bunner, D.L., Morris, E.R. 1988. Alterations of multiple cell membrane functions in L-6 myoblasts by T-2 toxin: an important mechanism of action. Toxicol. Appl. Pharmacol. 92, 113–121.

Burka, L.T., Doran, J., Wilson, B.J. 1982. Enzyme inhibition and the toxic action of moniliformin and other vinylogous β-ketoacids. Biochem. Pharmacol. 31, 79–84.

Charmley, L.L., Rosenberg, A., Trenholm, H.L. 1994. Factors responsible for economic losses due to *Fusarium mycotoxin* contamination of grain, foods and feedstuffs. In: Miller, J.D., Trenholm, H.L. (Eds.), Mycotoxins in Grain-Compounds other than Aflatoxin. Eagan, St. Paul, MN, pp. 471–486.

Charoenpornsook, K., Fitzpatrick, J.L., Smith, J.E. 1998. The effect of four mycotoxins on the mitogen stimulated proliferation of bovine peripheral blood mononuclear cells in vitro. Mycopathologia 143, 105–111.

Chelkowski, J. 1989. *Fusarium* mycotoxins, taxonomy and pathogenicity. In: Topics in Secondary Metabolites, vol. 2. Elsevier, Amsterdam.

Chen, L.Y., Tian, X.L., Yang, B. 1990. A study on the inhibition of rat myocardium glutathione peroxidase and glutathione reductase by moniliformin. Mycopathologia 111, 119–124.

Chu, F.S., Li, G.Y. 1994. Simultaneous occurrence of fumonisin B1 and other mycotoxins in moldy corn collected from the People's Republic of China in regions with high incidences of esophageal cancer. Appl. Environ. Microbiol. 60, 847–852.

Dacasto, M., Rolando, P., Nachtmann, C., Ceppa, L., Nebbia, C. 1995. Zearalenone mycotoxicosis in piglets suckling sows fed contaminated grain. Vet. Hum. Toxicol. 37, 359–361.

Diekman, M.A., Green, M.L. 1992. Mycotoxins and reproduction in domestic lifestock. J. Anim. Sci. 70, 1615–1627.

Dombrink-Kurtzman, M.A., Bennett, G.A., Richard, J.L. 1994. An optimized MTT bioassay for determination of cytotoxicity of fumonisins in turkey lymphocytes. J. Assoc. Off. Anal. Chem. 77, 512–516.

Dutton, M.F. 1996. Fumonisins, mycotoxins of increasing importance: their nature and their effects. Pharmacol. Ther. 70, 137–161.

Engelhardt, J.A., Carlton, W.W., Tuite, J.F. 1989. Toxicity of *Fusarium moniliforme* var. subglutinans for chicks, ducklings and turkey poults. Avian Dis. 33, 357–360.

Engler, K.H., Coker, R., Evans, I.H. 1999. A novel colorimetric yeast bioassay for detecting trichothecene mycotoxins. J. Microbiol. Meth. 35, 207–218.

Eppley, R.M. 1974. Sensitivity of brine shrimp (*Artemia salina*) to trichothecenes. J. Assoc. Off. Anal. Chem. 57, 618–620.

Fink-Gremmels, J. 1999. Mycotoxins: their implications for human and animal health. Vet. Quart. 21, 115–120.

Forgacs, J., Carll, W.T., Herring, A.S., Mahlandt, B.G. 1954. A toxic *Aspergillus clavatus* isolated from feed pellets. Am. J. Hyg. 60, 15–16.

Gathercole, P.S., Thiel, P.G., Hofmeyr, J.H. 1986. Inhibition of pyruvate dehydrogenase complex by moniliformin. Biochem. J. 233, 719–723.

Gelderblom, W.C.A., Jaskiewicz, K., Marasas, W.F.O., Thiel, P.G., Horak, R.M., Vleggar, R., Kriek, N.P.J. 1988. Fumonisins-novel mycotoxins with cancer promoting activity produced by *Fusarium moniliforme*. Appl. Environ. Microbiol. 54, 1806–1811.

Gelderblom, W.C.A., Kriek, N.P.J., Marasas, W.F.O., Thiel, P.G. 1991. Toxicity and carcinogenicity of the *Fusarium* moniliforme metabolite fumonisin B1, in rats. Carcinogenesis 12, 1247–1251.

Gelderblom, W.C.A., Marasas, W.F.O., Lebepe-Mazur, S., Swanefelder, S., Vessey, C.J., de la Hall, M.P. 2002. Interaction of fumonisn B1 and aflatoxin B1 in a short-term carcinogenesis model in rat liver. Toxicology 171, 161–173.

Groten, J.P., Tajima, O., Feron, V.J., Schoen, E.D. 1998. Statistically designed experiments to screen chemical mixtures for possible interactions. Environ. Health Persp. Suppl. 6, 1361–1365.

Grove, F.G., Mortimer, P.H. 1969. The cytotoxicity of some transformation products of diacetoxyscirpenol. Biochem. Pharmacol. 18, 1473–1478.

Hall, J.O., Javed, T., Bennett, G.A., Richard, J.L., Dombrink-Kurtzman, M.A., Côté, L.M., Buck, W.B. 1995. Serum vitamin A (retinal) reduction in broiler chicks on feed amended with *Fusarium* proliferatum culture material or fumonisin B1 and moniliformin. J. Vet. Diagn. Invest. 7, 416–418.

Hanelt, M., Gareis, M., Kollarczik, B. 1994. Cytotoxicity of mycotoxins evaluated by the MTT-cell culture assay. Mycopathologia 128, 167–174.

Harrison, L.R., Colvin, B.M., Greene, J.T., Newman, L.E., Cole, R.C. 1990. Pulmonary edema and hydrothorax in swine produced by fumonisin B1, a toxic metabolite of *Fusarium moniliforme*. J. Vet. Diagn. Invest. 2, 217–221.

Harvey, R.B., Kubena, L.F., Huff, W.E., Corrier, D.E., Rottinghaus, G.E., Phillips, T.D. 1990. Effects of treatment of growing swine with aflatoxin and T-2 toxin. Am. J. Vet. Res. 51, 1688–1693.

Holladay, S.D., Blaylock, B.L., Comment, C.E., Heindel, J.J., Luster, M.I. 1993. Fetal tyhmic atrophy after exposure to T-2 toxin: selectivity for lymphoid progenitor cells. Toxicol. Appl. Pharmacol. 121, 8–14.

Holt, P.S., DeLoach, J.R. 1988. Cellular effects of T-2 mycotoxin on two different cell lines. Biochim. Biophys. Acta 971, 1–8.

Holt, P.S., Buckley, S., DeLoach, J.R. 1987. Detection of the lethal effects of T-2 mycotoxin on cells using a rapid colorimetric viability assay. Toxicol. Lett. 39, 301–312.

Holt, P.S., Buckley, S., Norman, J.O., DeLoach, J.R. 1988. Cytotoxic effect of T-2 mycotoxin on cells in culture as determined by a rapid colorimetric bioassay. Toxicon 26, 549–558.

Hopmans, E.C., Murphy, P.A. 1993. Fumonisins: mycotoxins produced by *Fusarium moniliforme*. In: Bidlack, W.R., Omaye, S.T. (Eds.), Natural Protectants Against Natural Toxicants. Technomic Publishing, Lancaster, PA, pp. 61–78.

Huff, W.E., Doerr, J.A. 1981. Synergisms between aflatoxin and ochratoxin A in broiler chickens. Poult. Sci. 60, 550–555.

Huff, W.E., Harvey, R.B., Kubena, L.F., Rottinghaus, G.E. 1988. Toxic synergisms between aflatoxin and T-2 toxin in broiler chickens. Poult. Sci. 67, 1418–1423.

Ihara, T., Sugamata, M., Sekijiama, M., Okumura, H., Yoshino, N., Ueno, Y. 1997. Apoptotic cellular damage in mice after T-2 toxin-induced acute toxicosis. Nat. Toxins 5, 141–145.

Javed, T., Bennet, G.A., Richard, J.L., Dombrink-Kurtzman, M.A., Côté, L.M., Buck, W.B. 1993. Mortality in broiler chicks on feed amended with *Fusarium proliferatum* culture material or with purified fumonisin B1 and moniliformin. Mycopathologia 123, 171–184.

Javed, T., Dombrink-Kurtzman, M.A., Richard, J.L., Bennet, G.A., Côté, L.M., Buck, W.B. 1995. Serohematological alterations in broiler chicks on feed emended with *Fusarium proliferatum* culture material of fumonisin B1 and moniliformin. J. Vet. Diagn. Invest. 7, 520–526.

Joffe, A.Z. 1974. Toxicity of *Fusarium poae* and *F. sporotrichioides* and its relation to alimentary toxic aleukia. In: Purchase, I.F.H. (Ed.), Mycotoxins. Elsevier, Amsterdam, pp. 229–262.

Joffe, A.Z. 1978. *Fusarium poae* and *F. sporotrichioides* as principal agents of alimentary toxic aleukia. In: Wyllie, T.D., Morehouse, L.G. (Eds.), Mycotoxic Fungi, Mycotoxins, Mycotoxicoses. An Encyclopedic Handbook. Mycotoxicoses of Man and Plants: Mycotoxin and Control and Regulatory Aspects, vol. 3. Marcel Dekker, New York, Basel, pp. 21–86.

Joffe, A.Z. 1986. Detection of fusariotoxin-bioassay methods. In: Joffe, A.Z. (Ed.), Fusarium Species—Their Biology and Toxicology. Wiley, New York, pp. 136–152.

Johannisson, A., Björkhag, B., Hansson, W., Gadhasson, I.-L., Thuvander, A. 1999. Effects of four trichothecene mycotoxins on activation marker expression and cell proliferation of human lymphocytes in culture. Cell Biol. Toxicol. 15, 203–215.

Kasuga, F., Hara-Kudo, Y., Saito, N., Kumagai, S., Sugita-Konishi, Y. 1998. In vitro effect of deoxynivalenol on the differentiation of human colonic cell lines Caco-2 and T84. Mycopathologia 142, 161–167.

Knasmüller, S., Bresgen, N., Kassocie, F., Mersch-Sundermann, V., Gelderblom, W., Zöhrer, E., Eckl, P.M. 1997. Genotoxic effects of three *Fusarium* mycotoxins, fumonisin B1, moniliformin and vomitoxin in bacteria and in primary cultures of rat hepatocytes. Mutat. Res. 391, 39–48.

Kriek, N.P., Marasas, W.F., Steyn, P.S., van Rensburg, S.J., Steyn, M. 1977. Toxicity of a moniliformin-producing strain of *Fusarium moniliforme var. subglutinans* isolated from maize. Food Cosmet. Toxicol. 15, 579–587.

Krska, R., Josephs, R. 2001a. The state-of-the-art in the analysis of estrogenic mycotoxins in cereals. Fresenius J. Anal. Chem. 369, 469–476.

Krska, R., Baumgartner, S., Josephs, R. 2001b. The state of the art in the analysis of type-A and -B trichothecene mycotoxins cereals. Fresenius J. Anal. Chem. 371, 285–299.

Kubena, L.F., Edrington, T.S., Kamps-Holtzapple, C., Harvey, R.B., Elissalde, M.H., Rottinghaus, G.E. 1995. Influence of fumonisin B1, present in *Fusarium* moniliforme culture material, and T-2 toxin on turkey poults. Poult. Sci. 74, 306–313.

Kubena, L.F., Harvey, R.B., Buckley, S.A., Edrington, T.S., Rottinghaus, G.E. 1997. Individual and combined effects of moniliformin present in *Fusarium* fujikuroi culture material and aflatoxin in broiler chicks. Poult. Sci. 76, 265–270.

Kuiper-Goodman, T. 1995. Mycotoxins: risk assessment and legislation. Toxicol. Lett. 82/83, 853–859.

Kuiper, G.G.J.M., Carlsson, B., Grandien, K., Enmark, E., Haggblad, J., Nilsson, S., Gustafsson, J.-A. 1997. Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors α and β. Endocrinology 138, 863–870.

Langseth, W., Rundberget, T. 1998. Instrumental methods for determination of non-macrocyclic trichothecenes in cereals, foodstuffs and cultures. J. Chromatogr. A 815, 103–121.

Langseth, W., Kosiak, B., Clasen, P.-E., Gareis, M. 1997. Toxicity and occurrence of *Fusarium* species and mycotoxins in late harvested and overwintered grain from Norway, 1993. J. Phytopathol. 145, 409–416.

Langseth, W., Bernhoft, A., Rundberget, T., Kosiak, B., Gareis, M. 1999. Mycotoxin production and cytotoxicity of *Fusarium* strains isolated from Norwegian cereals. Mycopathologia 144, 103–113.

Lauren, D.R., Smith, W.A. 2001. Stability of fusarium mycotoxins nivalenol, deoxynivalenol and zearelenone in ground maize under typical cooking conditions. Food Addit. Cont. 18, 1011–1016.

Lautraite, S., Parent-Massin, D., Rio, B., Hoellinger, H. 1997. In vitro toxicity induced by deoxynivalenol (DON) on human and rat granulomonocytic progenitors. Cell Biol. Toxicol. 13, 175–183.

Le Guevel, R., Pakdel, F. 2001. Assessment of oestrogenic potency of chemcials used as growth promoter by in vitro methods. Hum. Reprod. 16, 1030–1036.

Leodux, D.R., Bermudez, A.J., Rottinghaus, G.E., Broomhead, J., Bennett, G.A. 1995. Effects of feeding *Fusarium* fujikuroi culture material, containing known levels of moniliformin, in young broiler chicks. Poult. Sci. 74, 297–305.

Lewis, C.W., Smith, J.E., Anderson, J.G., Freshney, R.I. 1999. Increased cytotoxicity of food-borne mycotoxins toward cell lines in vitro via enhanced cytochrome p450 expression using the MTT bioassay. Mycopathologia 148, 97–102.

Li, Y.C., Leodux, D.R., Bermudez, A.J., Fritsche, K.L., Rottinghaus, G.E. 2000. The individual and combined effects of fumonisin B1 and moniliformin on performance and selected immune parameters in turkey poults. Poult. Sci. 79, 871–878.

Lompe, A., Milczewski, K.E. 1979. Ein Zellkulturtest für den Nachweis von Mycotoxinen. Z. Lebensm. Unters. Forsch. 169, 249–254.

Madhyastha, M.S., Marquardt, R.R., Abramson, D. 1994. Structure-activity relationships and interactions among trichothecene mycotoxins as assessed by yeast bioassay. Toxicon 32, 1147–1152.

Marasas, W.F.O. 1991. Toxigenic Fusaria. In: Smith, J.E., Henderson, R.S. (Eds.), Mycotoxins and Animal Foods. CRC Press, London.

Marasas, W.F.O., Kellerman, T.S., Gelderblom, W.C.A. 1988. Leukencephalomalacia in a horse induced by fumonisin B1 isolated from *Fusarium moniliforme*. Onderstepoort J. Vet. Res. 43, 113–122.

Mayr, U. 1990. Tissue culture tests as an alternative to the uterotrophic assay in the detection of estrogenic active compounds. ALTEX 7, 5–16.

Mayr, U., Butsch, A., Schneider, S. 1992. Validation of two in vitro systems for estrogenic activities with zearalenone, phytoestrogens and cereal extracts. Toxicology 74, 135–149.

Mehmood, Z., Smith, A.G., Tucker, M.J., Chuzel, F., Carmichael, N.G. 2000. The development of methods for assessing the in vivo oestrogen-like effects of xenobiotics in CD-1 mice. Food Chem. Toxicol. 38, 493–501.

Miller, J.D., Taylor, A., Greenlagh, R. 1983. Production of deoxynivalenol and related compounds in liquid cultures. Can. J. Microbiol. 29, 1171–1178.

Morris, C.M., Li, Y.C., Leodoux, D.R., Bermudez, A.J., Rottinghaus, G.E. 1999. The individual and combined effects of moniliformin, supplied by *Fusarium* fujikuroi culture material, and deoxynivalenol in young turkey poults. Poult. Sci. 78, 1110–1115.

Morrison, E., Rundberget, T., Kosiak, B., Aastveit, A.H., Bernhoft, A., 2002. Cytotoxicity of trichothecenes and fusarochromanone produced by *Fusarium equiseti* strains isolated from Norwegian cereals. Mycopathologia, in press.

Musser, S.M., Gay, M.L., Mazzola, E.P., Plattner, R.D. 1996. Identification of a new series of fumonisins containing 3-hydroxypyridine. J. Nat. Prod. 59, 970–972.

Nagaraj, R.Y., Wu, W., Will, J.A., Valdivia, H.H., Lokuta, A., Vesonder, R.F. 1996. Moniliformin-a calcium channel blocker. Poult. Sci. Suppl. 75, 133.

Neiger, R.D., Johnson, T.J., Hurley, D.J., Higgins, K.F., Rottinghaus, G.E., Stahr, H. 1994. The short-term effect of low concentrations of dietary aflatoxin and T-2 toxin on mallard ducklings. Avian Dis. 38, 738–743.

Nijs, M.E., de Rombouts, F., Notermans, S. 1996. Fusarium molds and their mycotoxins. J. Food Safety 16, 15–48.

Nijs, M.E., de Rombouts, F., Notermans, S. 1997. Identification of hazardous *Fusarium* secondary metabolites occurring in food raw materials. J. Food Safety 17, 161–191.

Norred, W.P. 1993. Fumonisins-mycotoxins produced by *Fusarium* moniliforme. J. Toxicol. Environ. Health 38, 309–328.

Norred, W.P., Voss, K.A. 1994. Toxicity and role of fumonisins in animal diseases and human esophageal cancer. J. Food Protect. 57, 522–527.

Norred, W.P., Bacon, C.W., Porter, J.K., Voss, K.A. 1990. Inhibition of protein synthesis in rat primary hepatocytes by extracts of *Fusarium* moniliforme-contaminated corn. Food Chem. Toxicol. 28, 89–94.

Norred, W.P., Bacon, C.W., Plattner, R.D., Vesonder, R.F. 1991. Differential cytotoxicity and mycotoxin content among isolates of *Fusarium moniliforme*. Mycopathologia 115, 37–43.

Norred, W.P., Wang, E., Yoo, H., Riley, R.T., Merrill, A.H. 1992. In vitro toxicology of fumonisins and the mechanistic implications. Mycopathologia 117, 73–78.

Norred, W.P., Riley, R.T., Meredith, F.I., Bacon, C.W., Voss, K.A. 1996. Time- and dose–response effect of the mycotoxin, fumonisin

B1, on sphingoid base elevations in precision-cut rat liver and kidney slices. Toxicol. In Vitro 10, 349–358.

Norred, W.P., Plattner, R.D., Dombrink-Kurtzman, M.A., Meredith, F.I., Riley, R.T. 1997. Mycotoxin-induced elevation of free sphingoid bases in precision-cut rat liver slices: specifity of the response and structure activity relationships. Toxicol. Appl. Pharmacol. 147, 63–70.

Oldham, J.W., Allred, L.E., Milo, G.E., Kindig, G.E., Capen, C.C. 1980. The toxicological evaluation of the mycotoxins T-2 and T-2 tetraol using human fibroblasts in vitro. Toxicol. Appl. Pharmacol. 52, 159–168.

Panigrahi, S. 1993. Bioassay of mycotoxins using terrestrial and aquatic, animal and plant species. Food Chem. Toxicol. 31, 767–790.

Parent-Massin, D., Fuselier, R., Thouvenot, D. 1994. In vitro toxicity of trichothecenes on human haematopoietic progenitors. Food Add. Contam. 11, 441–447.

Parry, D.W., Jenkinson, P., McLeod, L. 1995. Fusarium ear blight (scab) in small grain cereals—a review. Plant Pathol. 44, 207–238.

Peraica, M., Radic, B., Lucic, A., Pavlovic, M. 1999. Toxic effects of mycotoxins in humans. Bull. Wrld. Health Org. 77, 754–766.

Pettersson, H. 1995. Trichothecene occurrence in European cereals—a review. Proceedings of the 17th German Mycotoxin Workshop. Landbauforschung Völkenrode Sonderheft 157, 155–158.

Pitt, J.I. 2000. Toxigenic fungi and mycotoxins. Br. Med. Bull. 56, 184–192.

Placinta, C.M., D'Mello, J.P.F., Macdonald, A.M.C. 1999. A review of worldwide contamination of cereal grains and animal feed with *Fusarium mycotoxins*. Anim. Feed Sci. Technol. 78, 21–37.

Porcher, J.-M., Lafarge-Frayssinet, C., Frayssinet, C., Nurie, A., Melcion, D., Richard-Molard, D. 1987. Determination of cytotoxic trichothecenes in corn by cell culture toxicity assay. J. Assoc. Off. Anal. Chem. 70, 844–849.

Prelusky, D.B., Hamilton, R.M.G., Foster, B.C., Trenholm, L.H., Thompson, B.K. 1987. Optimization of chick embryotoxicity bioassay for testing toxicity potential of fungal metabolites. J. Assoc. Off. Anal. Chem. 70, 1049–1055.

Prelusky, D.B., Rotter, B.A., Rotter, R.G. 1994. Toxicology of mycotoxins. In: Miller, J.D., Trenholm, H.L. (Eds.), Compounds other than Aflatoxin. Eagan, St. Paul, pp. 359–404.

Qureshi, M.A., Brundage, M.A., Hamilton, P.B. 1998. 4-beta, 15-diacetoxyscirpenol induces cytotoxicity and alterations in phagocytic and Fc-receptor expression functions in chicken macrophages in vitro. Immunopharmacol. Immunotoxicol. 20, 541–553.

Rafai, P., Tuboly, S., Bata, A., Tilly, P., Vanyi, A., Papp, Z., Jakab, L., Tury, E. 1995. Effect of various levels of T-2 toxin in the immune system of growing pigs. Vet. Rec. 136, 511–514.

Reubel, G.H., Gareis, M., Amselgruber, W.M. 1987. Cytotoxicity evaluation of mycotoxins. Mycotox. Res. 3, 85–96.

Riley, R.T., An, N.-H., Showker, J.L., Yoo, H.-S., Norred, W.P., Chamberlain, W.J., Wang, E., Merrill, A.H., Motelin, G., Beasley, V.R., Haschek, W.M. 1993. Alteration of tissue and serum sphinganine to sphingosine ratio: an early biomarker in pigs of exposure to fumonisin containing feeds. Toxicol. Appl. Pharmacol. 118, 105–112.

Riley, R.T., Wang, E., Merrill, A.H. 1994. Liquid chromatographic determination of sphinganine and sphingosine: use of the free sphinganine-to-sphingosine ratio as a biomarker for consumption of fumonisins. J. Assoc. Off. Anal. Chem. 77, 533–540.

Riley, R.T., Wang, E., Schroeder, J.J., Smith, E.R., Plattner, R.D., Abbas, H., Yoo, H.-S., Merrill, A.H. 1996. Evidence for disruption of sphingolipid metabolism as a contributing factor in the toxicity and carcinogenicity of fumonisins. Nat. Toxins 4, 3–15.

Robb, J., Kirkpatrick, K.S., Norval, M. 1982. Association of toxin-producing fungi with disease in broilers. Vet. Rec. 111, 389–390.

Robb, J., Norval, M. 1983. Comparison of cytotoxicity and thin-layer chromatography methods for detection of mycotoxins. Appl. Environ. Microbiol. 46, 948–950.

Robb, J., Norval, M. 1985. The use of a cytotoxicity test as a screening test for mycotoxins. In: Lacey, J. (Ed.), Trichothecene and other Mycotoxins. Wiley, New York, pp. 375–380.

Robb, J., Norval, M., Neil, W.A. 1990. The use of tissue culture for the detection of mycotoxins. Lett. Appl. Microbiol. 10, 161–165.

Robbana-Barnat, S., Lafarge-Frayssinet, C., Fraysinet, C. 1989. Use of cell cultures for predicting the biological effects of mycotoxins. Cell Biol. Toxicol. 5, 217–226.

Rosenstein, Y., Lafarge-Frayssinet, C. 1983. Inhibitory effects of *Fusarium* T2-toxin on lymphoid DNA and protein synthesis. Toxicol. Appl. Pharmacol. 70, 283–288.

Rotter, B., Thompson, B.K., Clarkin, S., Owen, T.C. 1993. Rapid colorimetric bioassay for screening of *Fusarium mycotoxins*. Nat. Toxicol. 1, 303–307.

Rotter, B., Prelusky, D.B., Pestka, J.J. 1996. Toxicology of deoxynivalenol (vomitoxin). J. Toxicol. Environ. Health 48, 1–34.

Saito, M., Ohtsubo, K., Umeda, M., Enemoto, M., Kurata, H., Udagawa, S., Sakabe, F., Ichinoe, M. 1971. Screening tests using HeLa cells and mice for the detection of mycotoxin-producing fungi isolated from foodstuffs. Jpn. J. Exp. Med. 41, 1–20.

Scossa-Romano, D.A., Bickel, R.E., Zweifel, U., Reinhardt, C.A., Lüthy, J.W., Schlatter, C.L. 1987. Fast and sensitive screening method for detection of thrichothecenes in maize by using protein synthesis inhibition in cultured fibroblasts. J. Assoc. Off. Anal. Chem. 70, 129–132.

Scott, P.M. 1991. Possibilities of reduction or elimination of mycotoxins present in cereal grains. In: Chelkowski, J. (Ed.), Cereal Grain. Mycotoxins, Fungi and Quality in Dying and Storage. Elsevier, Amsterdam, pp. 529–572.

Senter, L.H., Sanson, D.R., Corley, D.G., Tempesta, M.S., Rottinghaus, A.A., Rottinghaus, G.E. 1991. Cytotoxicity of trichothecene mycotoxins isolated from *Fusarium sporotrichoides* (MC-72083) and *Fusarium sambucinum* in baby hamster kidney (BHK-21) cells. Mycopathologia 113, 127–131.

Seo, J.-K., Kim, J.-C., Lee, Y.-W. 1996. Isolation and characterization of two new type C fumonisins produced by *Fusarium oxysporium*. J. Nat. Prod. 59, 1003–1005.

Shier, W.T., Abbas, H.K., Mirocha, C.J. 1991. Toxicity of the mycotoxins fumonisins B1 and B2 and *Alternaria alternata f*. sp. *Lycopersici* toxin (AAL) in cultured mammalian cells. Mycopathologia 116, 97–104.

Shier, W.T., Shier, A.C., Xie, W., Mirocha, C.J. 2001. Structure–activity relationships for human estrogenic activity in zearalenone mycotoxins. Toxicon 39, 1435–1438.

Shinozuka, J., Li, G., Kiatipattanasakul, W., Uetsuka, K., Nakayama, H., Doi, K. 1997. T-2 toxin-induced apoptosis in lymphoid organs of mice. Exp. Toxicol. Pathol. 49, 387–392.

Shokri, F., Heidari, M., Gharagozloo, S., Ghazi-Khansari, M. 2000. In vitro inhibitory effects of antioxidants on cytotoxicity of T-2 toxin. Toxicology 146, 171–176.

Smith, J.E., Lewis, C.W., Anderson, J.G., Solomons, G.L., 1994. Mycotoxins in human nutrition and health. Directorate General XII, Science, Research and Development, EUR 16048 EN.

Solfrizzo, M., Visconti, A., Avantaggiato, G., Torres, A., Chulze, S. 2001. In vitro and in vivo studies to assess the effectiveness of cholestyramine as a binding agent for fumonisins. Mycopathologia 151, 147–153.

Steyn, P.S. 1995. Mycotoxins, general view, chemistry and structure. Toxicol. Lett. 82/83, 843–851.

Sydenham, E.W., Thiel, P.G., Marasas, W.F.O., Shephard, G.S., van Schalkwyk, D.J., Koch, K.R. 1990. Natural occurrence of some *Fusarium* mycotoxins in corn from low and high oesophageal cancer prevalence areas of the Transkei, Southern Africa. J. Agric. Food Chem. 38, 1900–1903.

Thiel, P.G. 1978. A molecular mechanism for the toxic action of moniliformin, a mycotoxin produced by *Fusarium moniliforme*. Biochem. Pharmacol. 27, 483–486.

Thompson, W.L., Wannemacher, R.W. 1984. Detection and quantitation of T-2 mycotoxin with a simplified protein synthesis inhibition assay. Appl. Environ. Microbiol. 48, 1176–1180.

Thompson, W.L., Wannemacher, R.W. 1986. Structure-function relationships of 12,13-epoxytrichothecene mycotoxins in cell culture: comparison to whole animal lethality. Toxicon 24, 985–994.

Vesonder, R.F., Peterson, R.E., Plattner, R.D., Weisleder, D. 1990. Phytotoxic activity of selected water-soluble metabolites of *Fusarium* against *Lemna minor L*. (duckweed). Mycopathologia 11, 185–189.

Vesonder, R.F., Wu, W., Weisleder, D., Gordon, S.H., Krick, T., Xie, W., Abbas, H.K., McAlpin, C.E. 2000. Toxigenic strains of *Fusarium moniliforme* and *Fusarium proliferatum* isolated from dairy cattle feed produce fumonisins, moniliformin and a new $C_{21}H_{38}N_2O_6$ metabolite phytotoxic to *Lemna minor L*. J. Nat. Toxins 9, 103–111.

Visconti, A., Minervini, F., Lucivero, G., Gambatesa, V. 1992. Cytotoxic and immunotoxic effects of *Fusarium mycotoxins* using a rapid colorimetric bioassay. Mycopathologia 113, 181–186.

Visconti, A., Minervini, F., Solfrizzo, M., Bottalico, C., Lucivero, G. 1992. Toxicity of some *Fusarium* Section *Sporotrichiella* strains in relation to mycotoxin production. Appl. Environ. Microbiol. 58, 769–772.

Visconti, A., Solfrizzo, M. 1995. Visoltricin, a novel biologically active compound produced by *Fusarium tricinctum*. Food Add Contam. 12, 515–519.

Wang, E., Norred, W.P., Bacon, C.W., Riley, R.T., Merrill, A.H. 1991. Inhibition of sphingolipid biosynthesis by fumonisins. J. Biol. Chem. 266, 14486–144990.

Welshons, W.V., Rottinghaus, G.E., Nonneman, D.J., Dolan-Timpe, M., Ross, P.F. 1990. A sensitive bioassay for detection of dietary estrogens in animal feeds. J. Vet. Diagn. Invest. 2, 268–273.

Widestrand, J., Lundh, T., Pettersson, H., Lindberg, J.E. 1999. Cytotoxicity of four trichothecenes evaluated by three colorimetric bioassays. Mycopathologia 147, 149–155.

Williams, K.C., Blaney, B.J., Mageee, M.H. 1989. Responses of pigs fed wheat naturally infected with *Fusarium graminearum* and containing the mycotoxins 4-deoxynivalenol and zearalenone. Aust. J. Agric. Res. 40, 1095–1105.

Wu, W., Vesonder, R.F. 1997. Inhibition of gluconeogenesis in cultured chicken embryo hepatocytes by *Fusarium* metabolites. Nat. Toxins 5, 80–85.

Wu, W., Liu, T., Vesonder, R.F. 1995. Comparative cytotoxicity of fumonisins B1 and moniliformin in chicken primary cell cultures. Mycopathologia 132, 111–116.

Wu, W., Liu, T., Vesonder, R.F. 1995. Comparative cytotoxicity of fumonisin B1 and moniliformin in chicken primary cell culture. Mycopathologia 132, 111–116.

Yabe, T., Hashimoto, H., Sekijima, M., Degawa, M., Hashimoto, Y., Tashiro, F., Ueno, Y. 1993. Effects of nivalenol on hepatic drug-metabolizing activity in rats. Food Chem. Toxicol. 31, 573–581.

Yang, H.H., Aulerich, R.J., Helferich, W., Yamini, B., Chou, K.C., Miller, E.R., Bursian, S.J. 1995. Effects of zearalenone and tamoxifen on swine and mink reproduction. J. Appl. Toxicol. 15, 223–232.

Yates, I.E., Porter, J.K. 1982. Bacterial bioluminescence as a bioassay for mycotoxins. Appl. Environ. Microbiol. 44, 1072–1075.

Yates, I.E. 1986. Bioassay systems and their use in diagnosis of mycotoxicoses. In: Richards, J.L., Thurston, J.R. (Eds.), Diagnosis of Mycotoxicosis. Martinus Nijhoff, Dordrecht, Holland, pp. 333–381.

Yoo, H.S., Norred, W.P., Showker, J., Riley, R.T. 1996. Elevated sphingoid bases and complex sphingolipid depletion as contributing factors in fumonisin-induced cytotoxicity. Toxicol. Appl. Pharmacol. 138, 211–218.

Zhu, L.Z., Zia, Y.M., Yang, G.Q. 1982. Blood glutathione peroxidase activities of populations in Keshan disease affected and non-affected areas. Acta Nutr. Sin. 4, 229–233.