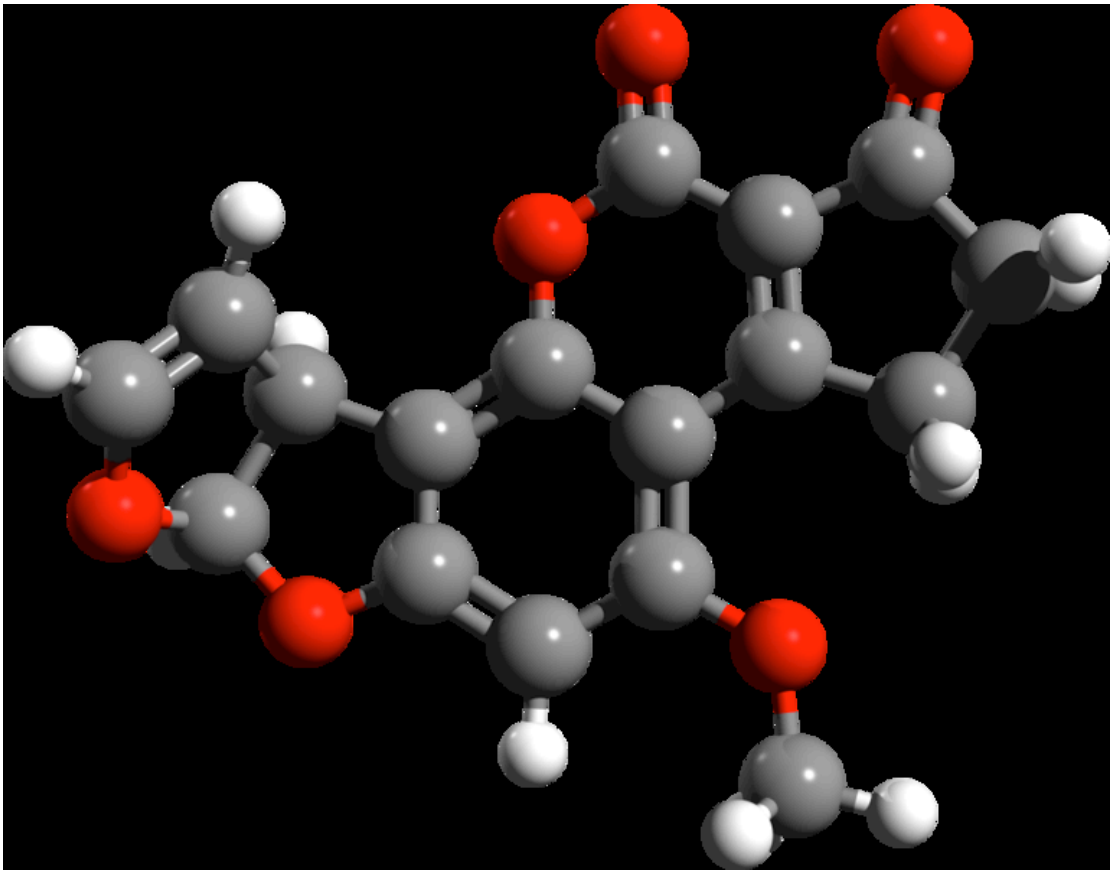
Airborne mycotoxin exposure



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Introduction

Fungi are ubiquitous in the environment, both indoors and outdoors; therefore, avoiding exposure to these organisms is impossible. In both residential settings and professional settings, risk assessment is needed in order to manage the risk involved with these organisms. The assessment of the amount of exposure to fungi is a very important issue in for example the food-producing industry. Two major concerns arise in relation to fungi: the first concern is exposure to the organisms themselves. Many fungi are pathogenic and can cause a number of illnesses; although many fungi are only opportunistic pathogens and can thus only harm immunocompromised persons, some fungi can cause severe illnesses in immunocompetent persons as well. The second concern is the presence of mycotoxins in the environment; mycotoxins are toxic secondary metabolites produced by many filamentous fungi, which can contaminate foods and feeds in the fields as well as in storage. 'Mycotoxin' is an artificial classification of pharmacologically active secondary fungal metabolites, which are toxic to vertebrates. (Bennett and Klich 2003) The reason it is an artificial classification is the difficulty of defining and classifying these compounds: mycotoxins show a very great diversity in chemical structure, biosynthetic origin, biological effect and wide number of fungal species that produce them. The toxicity of mycotoxins becomes apparent for example in the case of aflatoxin B1, which is considered the most potent mutagen produced by nature. (Shier 2011)

There are several exposure routes via which a person can become exposed to mycotoxins; the most important is the oral route, by eating contaminated food. The two other exposure routes are dermal, by touching the fungi themselves or objects contaminated with mycotoxins, and respiratory, by inhaling (parts of) fungi or their mycotoxins. The latter is the focus of this report; since many fungi reproduce by releasing spores into the air, airborne exposure to both the fungi themselves and to their mycotoxins is an important issue in the risk assessment of mycotoxins.

This report aims to investigate the risk of airborne exposure to mycotoxins by answering several questions:

1. What are the sources of the mycotoxins?
2. What are the relevant concentrations of these mycotoxins in the air?
3. What is the human exposure to these concentrations?
4. What is the dose of mycotoxins, which reaches the target organs?
5. What are the health effects of these mycotoxins?

The answers to these questions should provide the answer to the main research question: What is the risk of airborne exposure to mycotoxins?

This report specifically aims to investigate the airborne exposure component, as this is a less understood and studied component of the total mycotoxin exposure; most studies cover dermal or, more frequently, ingestion exposure.

The first part of this report will cover the producers of mycotoxins, fungi; it will detail which species pose the most risk, and where these are found. The second part will cover the mycotoxins themselves; which pose the greatest risk and why, their physiochemical properties, mode of action and target organs. The routes of exposure will also be briefly covered, the focus being on inhalation exposure. The third part will be an overview of studies into airborne exposure assessments, and will cover measurement and extraction techniques, and will discuss the conclusions drawn by authors. The final part will try to answer the main research question by answering the questions posed above and will try to come to a general conclusion about the main research question regarding airborne mycotoxin exposure.

Fungi & mycotoxins

Fungi are mostly multicellular, eukaryotic organisms and the diversity of the approximately 100,000 known and 1,5 million estimated fungal species is large enough to warrant their own kingdom. This diversity is partly responsible for the many different types of food and feed fungi can contaminate as decomposers of nature. Fungi as a kingdom, are divided into four phyla: the Chytridiomycota, the Zygomycota, the Ascomycota and the Basidiomycota. (Campbell and Reece 2002) Although fungi are not as important as human pathogens as they are as plant or insect pathogens, many fungi can pose a threat to human health. Two distinct health risks arise from fungal contamination; the first being illnesses caused by the invasion of and frank growth in the body by the fungi themselves, also collectively called mycoses. Mycoses can be relatively harmless, for example in the case of athlete's foot, but they can also be life threatening in the case of invasive aspergillosis. (Bennett and Klich 2003)

Figure 1: *Aspergillus flavus* on groundnuts Source: http://www.nri.org/research/foodsafety.htm

Most fungi that are known pathogens are opportunistic pathogens i.e. they can only harm immunosuppressed or immunocompromised individuals (e.g. *Candida albicans*) while only a few species are known to be primary pathogens, i.e. also able to cause illness in immunocompetent individuals (e.g. *Coccidioides immitis*). Abovementioned illnesses occur when fungi are permitted to grow frankly on or inside the body; although some remain localized to a certain part of the body (athlete's foot only affects the skin), the more serious fungal illnesses are systemic, i.e. have penetrated the

circulatory system and can cause serious health effects. (Bennett and Klich 2003)

The second risk from fungal contamination of food and feed does not require the fungi to grow on or inside the body; in these cases the secondary metabolites of fungi, mycotoxins, are the cause of the adverse health effects

Table 1. Overview of fungal metabolites with corresponding diseases, health concerns and species of origin, showing the variability of the mycotoxins and the broadness of the health risks. Source: Thrasher *et al.* (2009)

Metabolite	Disease	Organisms	Health Concerns
Gliotoxin	Invasive aspergillosis	*Aspergillus fumigatus, terres, flavus, niger, Trichoderma virens, Penicillium spp, Candia albican*	Immune toxicity, immune suppression, neurotoxicity
Aflatoxin B1; kojic acid; aspergillic acid; nitropopionic acid	Carcinogenesis	*Aspergillus flavus*	Liver pathology and cancer; immune toxicity; neurotoxicity
Fumigaclavines; fumitoxins; fumitermorgens; verruculogen; gliotoxin	Aspergillosis	*Aspergillus fumigatus*	Lung disease; neurotoxicity; tremors; immune toxicity
Ochratoxin A	BEN		Immunosuppression
	Urinary tract tumors;	*Aspergillus niger*	BEN
	Aspergillosis	*Penicillium verrucsum*	Lung disease
Ochratoxin A	Urinary tract	*Aspergillus ochraceus*	Nephropathology
Penicillic Acid; Xanthomegnin; Viomellein; Vioxanthin	Tumors		
Sterigmatocystin 5-methoxysterigmato cystin	Carcinogenesis	*Aspergillus versicolor*	Liver pathology and cancer
Chaetomiums; Chaetoglobosum A and C	Unknown	*Chaetomium globosum*	Cytotoxicity Cell division
Griseofulvin; Dechlorogrseofulvins	Unknown	*Memnoniella echinata*	Carcinogenesis? Reproductive toxin
Trichodermin; Trichodermo			Hypersensitivity? Protein synthesis inhibition
Mycophenolic acid	Unknown	*Penicillium brevicompactum*	Cytotoxic; mutagen
Botryodiploidin	Unknown	*Penicillium expansum*	Immune toxicity; cytotoxic;
Patulin; citrinin			
Chaetoglobosin			Tremors
Roquefortine C			
Verrucosidins	Unknown	*Penicillium plonicium*	Tremors, cytotoxicity;
Penicillic acid			Nephropathology
Nephrotoxic glyco-peptides			
Trichothecenes	Unknown	*Trichoderma species*	Trichothecene toxicity
Trichodermol			Immunotoxicity
Trichodermin			
Gliotoxin; Viridin			
Fumonisins	CNS birth defects	*Fusarium verticillioides* (aka *moniliforme*)	Neural tube defects in animals and humans
Spirocyclic	Pulmonary bleeding	*Stachybotrys chartarum*	Respiratory bleeding
Drimanes; roridin			Protein synthesis inhibition
Satratoxins (F, G, H)			Neurotoxicity
Hydroxyroridin E			Cytotoxicity
Verrucarin J			Immune toxicity
Trichodermin			
Dolabellanes			
Altrones B, C;			
Stahybotrylactams			

and hence these adverse health effects are called mycotoxicosis. Because the fungi do not need to grow or even to be present at the body, these adverse health effects are treated in the same manner as for example poisoning by exposure to pesticides. (Bennett and Klich 2003) In general there are no treatments for mycotoxicosis other than supportive therapy, such as adjusted diet and hydration; therefore prevention of exposure to mycotoxins is preferable. Studies have been performed to assess the risk from exposure via ingestion, but airborne and dermal exposure studies into mycotoxins are much more limited in number.

Although fungi are economically very important for the production of food (cheeses and fermentation of alcoholic beverages for example) they are also of great economical importance because of their decomposing potential; they are responsible for great losses in food and feed. Several different genera of fungi produce mycotoxins, which can be a hazard for human health; among them very common food and feed spoiling species. (Paterson and Lima 2010)

Due to the aforementioned difficulty in classification of mycotoxins according to a single criterion, the following section will contain an alphabetically arranged detailing of the most important mycotoxins, a short summary of their characteristics and the species that produce them; table 1 presents an overview of these characteristics.

Aflatoxins

Aflatoxins are difuranocoumarin derivatives, which are produced by a polyketide pathway in *Aspergillus flavus* and *Aspergillus parasiticus*. These toxins were first isolated after the 'Turkey X disease', caused by batches of peanuts in chicken feed, which were contaminated by *Aspergillus flavus*. There are four major forms of this toxin, named after the color of light they emit when excited by UV light: B1, B2, G1 and G2 (B being blue and the G being green colors); although more variants exist, these four are the most important. There are about a dozen less common variants, such as the P1, Q1, B2a and G2a, but for the purpose of risk assessment, these are relatively unimportant. Since *Aspergillus flavus* is a major contaminant in agriculture, contaminating various different foodstuffs such as cereals and rice.

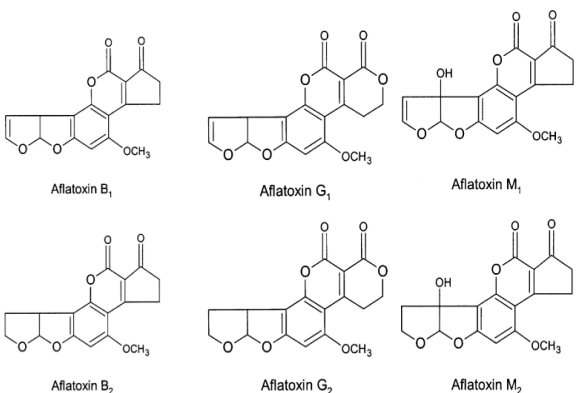

Figure 2. Chemical structures of aflatoxins B, G and M.
Source: Zain *et al.* (2011)

Because of the significance of *Aspergillus* species as a food contaminant, aflatoxins are of great interest in assessing risk to mycotoxin exposure.

Aspergillus flavus is not the only

strain of *Aspergillus* producing aflatoxins, but it is the most common strain and thus of most interest of researchers. There is much difference in quantity of aflatoxin production between the different *Aspergillus flavus* strains; only about half of the *Aspergillus flavus* produce aflatoxin, but those that do can often produce more than 10^6 ug/kg. LD_{50} values for acute aflatoxin exposure are hard to summarize and extrapolate because of the large differences in susceptibility of the different animal species; results of animal studies range from 0.5 – 10.0 mg/kg bodyweight. (Bommakanti and Waliyar 2000) However, estimated LD_{50} values for humans range from 10-20 mg/kg bodyweight for adults; these values have been extrapolated from animal experiments and a case study. The case study involved an Indian outbreak of hepatitis in 1974, in which 100 people died as a result of aflatoxin intoxication via ingestion of contaminated maize. It was later estimated that some adults might have eaten 2-6 mg of aflatoxin in a single day. (Braicu and others 2010) Chronic toxicity data suggest aflatoxin B1 is the most potent carcinogen known and chronic exposure has also been shown to cause immunosuppression. (Bennett and Klich 2003) Toxicity of aflatoxin is based on the conversion of aflatoxin by cytochrome P450 enzymes into a reactive 8,9 epoxide form. This form is able to bind to guanine in DNA and proteins at the N^7 position, and causing GC-TA transversions in DNA, explaining aflatoxins carcinogenic potential in many studies; they concluded a high correlation between aflatoxin and the number of DNA adducts *in vivo*. (Bennett and Klich 2003) Several studies link airborne exposure to aflatoxin B1 to cancer incidence and the IARC has classified aflatoxin B1 as a class I carcinogen. (Bennett and Klich 2003)

Citrinin

First isolated from *Penicillin citrinum* before World War II, citrinin was later also found in many other *Penicillin* species. Two fungi strains used to make pigments in industry, *Monascus ruba* and *Monascus purpureus*, have also been found to produce citrinin, along with several *Aspergillus* species.
Citrinin has acute nephrotoxicity in all tested animal species although there are large differences in susceptibility. Whereas citrinin has a LD_{50} value of 57 mg/kg bodyweight in ducks, rabbits have an LD_{50} value of 134 mg/kg bodyweight. Citrinin is thermally unstable in aqueous solution, and has antibiotic properties against gram-positive bacteria; however, it is not used as an antibiotic because of the high nephrotoxicity.

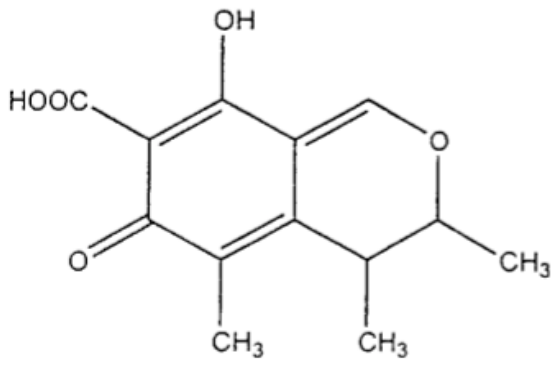

Figure 3. Citrinin. Source: Bennett *et al.* (2003)

Citrinin is frequently found in food and feed with ochratoxin A, and this

combination seems to be involved in Balkan endemic nephropathy although how this occurs is still unknown. (Flajs and Peraica 2009) However, these two compounds seem to depress RNA syntheses, as researched in rat and mouse models. (Bennett and Klich 2003) Food colored by *Monascus* pigments is known to contain citrinin and it is regularly found in other food, such as wheat, oats, corn and rice, but the implications of this for human health are unknown. (Bennett and Klich 2003)

Ergot alkaloids

The ergot alkaloids are a family of many different mycotoxins; chemically they belong to the family of indole alkaloids, and they are derived from tetracyclic ergotine ring systems. The structure found in all ergot alkaloids, lysergic acid, was first isolated in 1934. The structure as shown in figure 4, is the basis of all ergot alkaloids; the complete group of mycotoxins consists of this structure, with different moieties substituted on the numbered positions. Ergot alkaloids are produced by *Claviceps* species, a common contaminant of grasses, and also of grain. Therefore, the impact this fungus has on the human food supply has been major; however, current methods of grain cleaning are more than sufficient to prevent contamination of grain with *Claviceps*. Ergotism has all but been eliminated in humans, but it still remains a veterinary problem. (Bennett and Klich 2003)

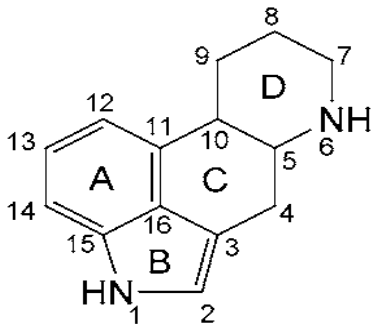

Figure 4. Tetracyclic ergoline ring system, the basic structure for all ergot alkaloids. Source: Wallwey *et al.* (2011)

The exposure to the alkaloids of *Claviceps* causes two different types of ergotism (or St. Anthony's Fire): the gangrenous form, which affects the blood supply to the extremities and the convulsive form, which affects the central nervous system. Another use of ergot alkaloids involves their many therapeutic properties if they are given in the correct doses. Because ergot alkaloids cause smooth muscle contractions it is also used as an arbortifacient and uterine contraction accelerant. The most famous ergot alkaloid is LSD (Lysergic Acid Diethylamide) the famous recreational drug, but ergot alkaloids are also used in for example treatment of migraine and treatments against Parkinsonism. (Bennett and Klich 2003)

Fumonisins

Fumonisins were first described and characterized in 1988; the most abundantly produced form is fumonisin B1 (see figure 5). This mycotoxin is synthesized by condensation of the amino acid alanine into an acetate-derived precursor. (Bennett and Klich 2003) Fumonisins are produced by many *Fusarium* species, amongst others *Fusarium verticillioides*, *F.*

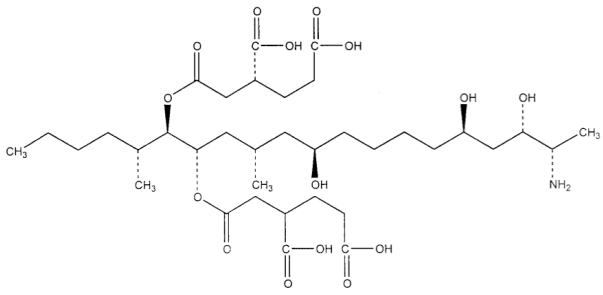

Figure 5. Fumonisin B1. Source: Bennett *et al.* (2003)

proliferatum, *F. nygamai* as well as by *Alternaria alternata*. Fumonisins act by interfering with spingolipid metabolism and they can cause several animal diseases. Fumonisins are also linked with esophageal cancer: the occurrence of Fumonisin B1 on food has been linked to higher incidents of esophageal cancer in certain regions of South Africa. (Bennett and Klich 2003) Acute intoxication by Fumonisins can cause several symptoms: abdominal pain, borborygmus and diarrhea. The International Agency for Research on Cancer (IARC) has classified fumonisins as a class 2B compound (possibly carcinogenic to humans). There has been some debate about the exposure to fumoninins by food, because fumonisins are difficult to detect due to their hydrophilic nature; many extraction methods fail to extract them. (Bennett and Klich 2003) Mostly aqueous methanol and aqueous acetonitrile are used to successfully extract fumonisins from a sample.

Ochratoxin

Ochratoxins are produced by *Aspergillus ochraceus* and several other *Aspergillus* species, among which is also *Aspergillus niger*; this last strain is of particular importance because of the increased risk. *Aspergillus niger* is used in the industrial production of certain enzymes and citric acid for human consumption; therefore the potential of human exposure is vastly increased. (Bennett and Klich 2003) Ochratoxins were discovered in a large screening specifically to find new mycotoxins in 1965. Afterwards, they were discovered in samples of barley, oats and coffee beans. Ochratoxins are potent nephrotoxins and the kidneys are the primary target organs. The importance of ochratoxins in *Aspergillus* species rivals the importance of the aflatoxins, which are produced by the same species. As stated before ochratoxins are nephrotoxic, which becomes clear in all animal experimentation; they are very likely to be nephrotoxic to humans too, since ochratoxins have a longer half-life in humans than they do in animals. Further indications of toxicity of ochratoxins are hepatotoxicity and immunosuppression. They are also potent teratogens and carcinogens, increasing the risk to testicular cancer. (Bennett and Klich 2003; Paterson and Lima 2010) The mechanism of action of ochratoxins is based on the disturbance of enzymes involved in the

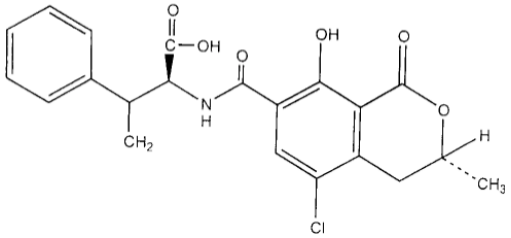

Figure 6. Chemical structure of ochratoxin A. Source: Bennett *et al.* (2003)

metabolism of fenylalanine. They also inhibit mitochondrial ATP production and stimulate lipid peroxidation. Ochratoxins have been detected in human milk and animal blood and tissues, and in pork for human consumption. Ochratoxins are suspected to be the causative agent of Balkan nephropathy and several exposure assessments have been done, leading to the limit set by the European Union Scientific Committee of 5 ng/kg bodyweight per day. The IARC has deemed ochratoxins a class 2B compound, or a possible human carcinogen. (Bennett and Klich 2003; Paterson and Lima 2010)

Patulin

Patulins are produced by many different fungal species and were first isolated from *Penicillium patulum* (now called *Penicillium griseofulvum*) in the 1940s.

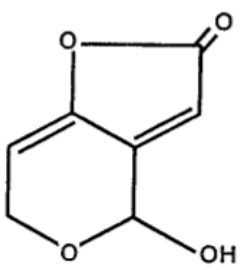

Figure 7. Patulin. Source: Bennett *et al.* (2003)

The first use of patulin was a test as an antibiotic but it became apparent that it was also very toxic to plants and animals. Thus, in the 1960s it was reclassified as a mycotoxin. For general human exposure, the soft blue mold found on fruits is the most important exposure source. Patulin is not the most potent of toxins: at (very) high laboratory concentrations it exhibits toxicity but evidence of natural poisoning is indirect and inconclusive. Nevertheless, the WHO has set a maximum TDI of 0.4 mg/kg bodyweight per day for patulin; patulin's main use today is in the experimental research into the biochemistry of polyketide biosynthesis pathways. (Bennett and Klich 2003; Paterson and Lima 2010)

Sterigmatocystins

Sterigmatocystins are produced by many *Aspergillus* species like *Aspergillus vesicolor*, *A. chevalieri*, and *A. ruber*, amongst others. Several other fungi produce sterigmatocystins, such as *Penicillium, Bipolaris, Chaetomium* and *Emiricella*. Sterigmatocystins are biogenic precursor compounds to aflatoxin B1, hence their equally potent carcinogenic, mutagenic and teratogenic properties. (Veršilovskis and de Saeger 2010) Although the human health hazard of sterigmatocystin is unknown, animal studies indicate the abovementioned dangers; it is also a potent causative agent of acute liver toxicity. Although the producing fungi are widespread, sterigmatocystins are rarely found; therefore, sterigmatocystins are of less importance than most other mycotoxins as far as human risk assessment is concerned. However,

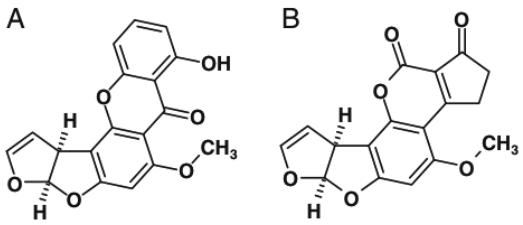

Figure 8. Structures of sterigmatocystin (A) and aflatoxin B1 (B). Source: Versilovskis *et al.* (2010)

they do serve another purpose in research: they are heavily used as model compounds in cancer induction studies. (Veršilovskis and de Saeger 2010)
The acute toxic effects of sterigmatocystins are much like aflatoxin, causing kidney and liver toxicity. The LD_{50} values of sterigmatocystin range from 220 mg/kg bodyweight in female rats to 800 mg/kg bodyweight in mice.
The mode of action is suspected to be identical to aflatoxin, because of the structural similarities between the two compounds: the mutagenic properties are most likely caused by the binding to the same N^7 guanine adduct of DNA molecules just like aflatoxin. (Veršilovskis and de Saeger 2010)

Trichothecenes

The trichothecenes are a family of more than 60 sesquiterpenoid metabolites and they are produced by several genera of fungi: *Fusarium*, *Myrothecium*, *Phomopsis*, *Stachybotrys*, *Trichodema*, *Trichothecium* and some others. All trichothecenes have the same basic 12,13-epoxytrichothecene skeleton to which a olefinic bond binds various side chains. They are commonly found in food and feed and skin contact can cause dermatitis; ingestion or other exposure can cause hemorrhaging and vomiting. They come in two main groups: the macrocyclic trichothecenes and the non-macrocyclic trichothecenes; the cyclic compounds have a macrocyclic ester bridge between C-4 and C-15.

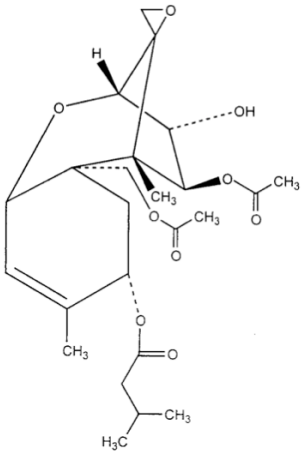

Figure 9. T-2 Toxin. Source: Bennett *et al.* (2003)

The non-macrocyclic group is further divided into group A, which have a hydrogen or ester type sidegroup to the C-8 position and group B, which have a ketone group at the C-8 position. The A group includes T-2 toxin, neosolaniol and diacetoxyscirpenol; the B-group includes fusarenon-x, nivalenol, and deoxynivalenol. (Bennett and Klich 2003; Paterson and Lima 2010) The main producers of these toxins are *Fusarium* species, and the mode of action is an extremely potent inhibition of eukaryotic protein synthesis; the 12,13-epoxy group is essential for toxicity in these compounds. The most intensively studied trichothecenes produced by *Fusarium* are diacetoxyscirpenol, deoxynivalenol and T-2 toxin. There are many symptoms caused by these toxins in vertebrates; for example, deoxynivalenol is also called vomitoxin, after one of the more visible symptoms. Although the mechanisms are poorly understood, all are likely caused by the protein synthesis inhibition. (Bennett and Klich 2003; Paterson and Lima 2010)
The macrocyclic trichothecenes are produced by *Myrothecium*, *Stachybotrys* and *Trichothecium* for the most part. A mixture of Verrucarin A and B, called glutinosin was originally identified as a antimicrobial agent, not a mycotoxin, as is the case with more types of antibiotics. The line between a mycotoxin

and an antibiotic is sometimes very thin, as is the case with penicillin. (Bennett and Klich 2003; Shier 2011) *Stachybotrys chartarum* also produces toxins of the trichothecene family: satratoxins, roridins, verrucarins and atranones are among them. *Stachybotrys* species causes the condition stachybotryotoxicosis, which was earlier considered only a horse disease; in recent years however, more cases became apparent in humans as well, increasing the interest in this fungus as well as in its toxins. (Bennett and Klich 2003)

Zearalenone

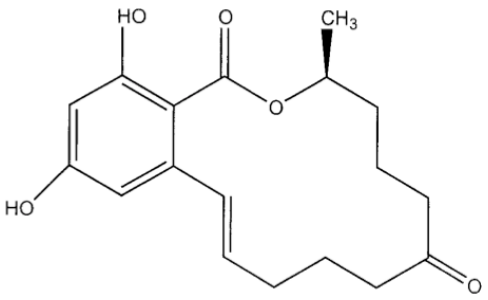

Figure 10. Zearalenone. Source: Bennett *et al.* (2003)

Zearalenone (6-[10-hydroxy-6-oxo-*trans*-1-undecenyl]-B-resorcyclic acid lactone) is produced by *Fusarium graminearum*; it is also known as F-2 toxin. But the name 'toxin' is somewhat of a misnomer; while zearalenone is biologically potent, it is hardly toxic. It is more an estrogen receptor agonist because of its resemblance to 17β-estradiol; more accurate names are non-steroidal estrogen or mycoestrogen. It is synthesized through a polyketide pathway by *Fusarium graminearum*, *F. culmorum*, *F. equiseti* and *F. crookwellense*; all these species are common contaminants of cereal crops. The reduced form of zearalenone, zearalenol, has increased estrogenic activity and is patented as an oral contraceptive or prescribed to postmenopausal women to relieve their symptoms. (Bennett and Klich 2003; Maragos 2010)

As stated, the toxicity of zearalenone is relatively low: it has an LD_{50} of more than 10,000 mg/kg bodyweight in female rats and 5,000 mg/kg bodyweight in guinea pigs. But its potency as a mycoestrogen is much greater: as little as 1 ug/kg bodyweight can cause uterogenic responses in swine. (Bennett and Klich 2003)

Risk to human populations is minimal, according to reviews of epidemiological data; but because of the high potency of this compound it is not wise to underestimate the possible hidden effects of zearalenone. (Bennett and Klich 2003; Maragos 2010)

Airborne mycotoxin exposure in literature

The next section details a summary of literature, consisting of reviews as well as primary literature describing studies into several mycotoxins and a possible exposure via inhalation.
The literature was obtained in three categories: primary literature covering exposure measurements in home or work environments related to airborne mycotoxin exposure, the primary literature covering mouse/rat experiments investigating (airborne) mycotoxin exposure and the reviews about these primary sources.

Sources of airborne mycotoxins

In essence, every place that can support fungal growth can be a source of mycotoxins. Table 2 shows the most important mycotoxins along with the fungi that most commonly produce them; however, not every place that has fungal growth also has mycotoxins. Fungi do not always produce mycotoxins; this is a difficulty in determining mycotoxin exposure using the quantities of fungi present. (Paterson and others 2010) An important aspect in the risk assessment of airborne mycotoxin concentrations is the location of measurements; obviously, some locations have more chance for high concentrations than others. The measuring locations are therefore variable, although several locations return more frequently: grain farms (Halstensen and others 2006a; Halstensen and others 2006b; Tangni and Pussemier 2007) and moldy buildings in some shape or form. (Bloom and others 2007; Brasel and others 2005b; Sawane and Saoji 2005) Laboratory settings appear in several studies, but these are mostly studies utilizing cultured fungi, not studies directly measuring concentrations in air. (Fischer and others 2006; Moularat and Robine 2008; Panaccione and Coyle 2005) A more unlikely source of airborne mycotoxin exposure was investigated by Pauly *et al.* (2010). They performed a review of observations about lung inflammation in smokers and its possible causes by mycotoxins. They did several observations indicating the presence of mycotoxins in tobacco, and cigarettes from all researched

Table 2. Fungi with their most commonly associated mycotoxiins.
Source: Paterson *et al. (2010)*

Fungus	Mycotoxin
Aspergillus carbonarius, *A. ochraceus*	Ochratoxin A (OTA)
A. flavus	Aflatoxin B_1 (AFB_1), AFB_2
A. parasiticus	AFB_1, AFB_2, AFG_1, AFG_2
A. niger	OTA, fumonisins (FUMs)
A. terreus, *A. clavatus*	Patulin
Byssochlamys fulva, *B. nivea*	Patulin
Fusarium cerealis, *F. poae*	Nivalenol (NIV)
F. culmorum, *F. graminearum*	NIV, deoxynivalenol (DON)
F. equiseti	Zearalenone
F. sporotrichioides	T-2 toxin
F. verticillioides (= *F. moniliforme*)	Fumonisin B_1 ($FUMB_1$)
Penicillium expansum, *P. roqueforti*	Patulin
P. verrucosum	OTA

cigarette brands contained mold and mycotoxins, mostly aflatoxin. The cause of these microbial contaminations is most likely the curing phase of tobacco production, during which molds have a chance to develop in the tobacco. In short, cigarette smoking should be considered as a new source of exposure to mycotoxins and be taken into account when performing risk assessments. (Pauly and others 2010)

Most samples however, are collected in the form of dust samples, either settled or airborne dust, by some type of vacuum device. Several studies used pieces of the actual fungi, and dissolved these to extract the mycotoxins; Sawane *et al.* for example, cultured the fungal samples on agar and cut out pieces to extract the mycotoxins. (Sawane and Saoji 2005) Pannaccione *et al.* used a similar method; they obtained the conidia from the sampled fungi and dissolved these in a methanol-water mixture to extract the mycotoxins. (Panaccione and Coyle 2005) The downside of these methods is the variability of fungal mycotoxin production; the mycotoxin concentrations have to be deduced from the quantity of fungi sampled. Therefore, these methods are more suited for qualitative studies.

Panaccione *et al.* (2005) also investigated the ability of *Aspergillus fumigatus* as vehicle for mycotoxin exposure. The conidia of *A. fumigatus* contain high levels of ergot alkaloids, and since these conidia are very light they facilitate fast aerosolization of the alkaloids; this could possibly be a source for mycotoxins without the need for mycotoxins to be very volatile. (Panaccione and Coyle 2005) A large review by Thrasher *et al.* (2009) also concluded that mycotoxins in damp indoor environments can become airborne and do so on larger particles (spores, hyphae fragments) as well as on smaller particles and in bulk and dust. Multiple kinds of mycotoxins are prevalent in water-damaged buildings. There is sufficient evidence from multiple authors that come to the same conclusion. (Thraser and Crawly 2009) Furthermore, a correspondence by Straus *et al.* (2006) concludes that *Stachybotrys chartarum* grows in buildings where people have health problems; *S. chartarum* produces macrocyclic trichothecene mycotoxins and these are inhaled and taken up by the people in these buildings. The only question the authors posed was the concentration of mycotoxin to accumulate in these people; they did not have sufficient data to state with certainty that the concentrations of mycotoxins taken up by these people are enough to cause the health problems. (Straus and Wilson 2006)

Concentration of mycotoxins in the air

To measure mycotoxin concentrations in the air, several methods have been developed; however, many of these methods are still ‘experimental’, meaning there is still some validation necessary. Several studies therefore describe methods to measure airborne concentrations and methods to extract the mycotoxins from the samples.

Bloom *et al.* (2007) developed an HPLC-MS/GC-MS based method for the direct detection of several mycotoxins. (Bloom and others 2007) Brasel *et al.* (2005) compared an ELISA based test to HPLC methods and found the ELISA to be more sensitive. (Brasel and others 2005a)
Sampling was generally performed using vacuum pumps/air samplers (table 2); in studies more focused on obtaining qualitative results, obtaining spores or culturing of certain species of fungi was performed.
Extraction was typically done using methanol, combined with water or other organic solvents like dichloromethane. In two instances, an acetonitrile/water mixture was used in extraction.
In most studies, High Pressure Liquid Chromatography (HPLC) was used in analysis, sometimes in combination with Mass Spectrometry (MS). Another widely used analytic method was the Gas Chromatography (GS) sometimes again in combination with MS; exceptions are present however (table 2). Immunoassays in the form of Enzyme-linked Immunosorbant Assay (ELISA) were used in analysis of *Stachybotrus chartatum*, combined with HPLC.
There are widely varying limits of detection (LODs), both for the sampling and analysis steps; besides the variation, the units differ between papers: some only provide (pico) grams, others calculate the LODs in (nano) grams per milliliter or cubic meter. A third approach is calculating the (nano) grams of mycotoxin per kilogram of dust sample collected. The LODs also vary across the different toxins measured and can range from as little as 0.2 pg (Bloom and others 2007) to 100 ug. (Halstensen and others 2006a)
There are several papers detailing new methods for identification and characterization of mycotoxins; Fischer *et al.* (2006) developed a method using Fourier Transform Infrared Spectroscopy (FT-IR) to identify different fungal species by their (secondary) metabolites. This method could also be employed to directly identify these metabolites, thus possibly increasing speed and improving cost-efficiency over other methods. (Fischer and others 2006) Real-Time Polymerase Chain Reaction (RT-PCR) has also been suggested as a fast and cost-effective way to identify fungal species. An advantage of this method is the possibility of this method to perform the PCR on any part of the fungus that contains DNA and the precision associated with this method. (Halstensen and others 2006a) In another paper by the same author, the trichodiene synthase gene (tri5) was investigated with Quantitative PCR (QPCR) and compared to measurements of several mycotoxins that were measured using Gas Chromatography coupled to Mass Spectrometry (GC-MS). A strong correlation was found between the two measurement methods, and the authors suggested that the PCR method could be utilized to improve health risk assessments related to mycotoxins. (Halstensen and others 2006b) Another study into measuring methods was performed by Moularat *et al.* (2008) using a device created by the researchers in combination with HPLC. This method enabled them to directly measure airborne mycotoxins but not just the mycotoxins the researchers first intended

(trichothecenes) but also sterimatocystin, deoxynivalenol and ochratoxin. Furthermore, the authors stated that other polar molecules might also be measurable by this method, adding to the usability of the method. Although this study produced quantitative data about airborne mycotoxins, this was only data obtained in laboratory setting, not in actual measurements outside the lab. (Moularat and Robine 2008)

Wang *et al.* (2008) described the optimization of a method of determining airborne mycotoxins, based on HPLC; their method could simultaneously quantify six kinds of mycotoxins. This study was the only study, which produced quantitative results, measuring in a poultry house. They found the following concentrations (mean +/- SD); for aflatoxin G2, B1 and zearalenone 0.189+/-0.024, 0.080+/-0.003 and 2.363+/-0.030 ng/m^3 air, respectively. For ochratoxin A a concentration of 8.530 (n=1) was found, while two other mycotoxins, aflatoxin B1 and G2, produced no positive samples. (Wang and others 2008)

Airborne mycotoxin exposure is a relatively unexplored field, as shown by Thacker (2004); as of 2004, no studies had been performed to investigate direct exposure to airborne mycotoxins. (Thacker 2004)

In conclusion, studies providing quantitative data about airborne mycotoxin concentrations are rare; most studies describe method testing, or have performed qualitative testing. There have been studies researching ways to correlate airborne concentrations to other data, (Fischer and others 2006) but these results have to be validated by further research to gain more credibility.

Exposure to airborne mycotoxins

The lack of direct airborne exposure studies was addressed by Brasel *et al.* (2005) when they published two papers concerning airborne mycotoxin exposure; (Brasel and others 2005a; Brasel and others 2005b) With these two papers, they demonstrated that macrocyclic trichothecene mycotoxins could become airborne in indoor environments with fungal infestations. The exposure risk from these airborne mycotoxins is as of yet unknown because quantitative data was not obtained by these studies. (Brasel and others 2005b)

A study of ergot alkaloids by Panaccione *et al.* (2005) focused on the mycotoxins on conidia. They found that the there is exposure risk posed by these conidia, and that these exposure risks depend on the concentration of mycotoxins on each conidium, the number of conidia and the toxicity of the toxins involved. (Panaccione and Coyle 2005)

A preliminary study by Tangni *et al.* published in 2007 assessed the mycotoxin content of grain dusts from cereal storage facilities. More than a dozen mycotoxins were measured; grain dust was found to contain very high quantities of mycotoxins. However, this study did not perform measurements directly in the air: they measured only in settled dust samples. They recommended that studies be performed to calculate uptake as well as intake

for workers in these facilities and that subsequently Tolerable Daily Intake (TDI) values be determined. (Tangni and Pussemier 2007)
Exposure to airborne mycotoxins has not been the subject of many studies; most studies measure in (settled) dust, which gives some indication, but an exposure assessment cannot be reliably extracted from these results.

Health effects of airborne mycotoxins

There is a great variety in mycotoxins covered by the papers in tables 3 and 4. It is this variety that complicates matters concerning health effects. There is a lot of difference in properties and toxicity of mycotoxins and ideally a risk assessment should be made for each one.
Possible health risks in exposure to airborne mycotoxins can include increased (lung) cancer risk since it is known that some mycotoxins have genotoxic effects. (Bünger and others 2004) Studies suggest mycotoxins may reduce immunity (Bennett and Klich 2003) and early cancer onset has also been linked to mycotoxin exposure. (Krska and Molinelli 2007; Marin and others 2002)
Comparison of the ergot alkaloids to similar substances suggests that very high quantities need to be inhaled for direct health effects to occur. Chronic, low dosage exposures seem to be more likely and could produce less remarkable effects like disruptions in sleep-wake cycles, depressions and blood-pressure changes. (Panaccione and Coyle 2005) Complications could also include inflammatory responses to mycotoxins, as studied by Pauly *et al.* in 2010.
However, not only single mycotoxins should be considered; the synergistic and antagonistic effects of simultaneous exposure to several mycotoxins, which is common, are unknown, and should also be considered. (Bünger and others 2004) Furthermore, synergism between mycotoxins and other pollutants might also exacerbate complications for human health. (Thrasher and Crawley 2009)
Amuzie *et al.* (2008) compared the effects of oral exposure to nasal exposure, where nasal exposure served as a model for airborne exposure in mice. Their conclusions suggested that deoxynivalenol (DON) is distributed to a greater extend and evoked more toxicity in the target tissues when the mice were exposed via airborne administration than via oral administration. Authors cite another study in which similar results were obtained; together this could mean that DON and possibly other trichothecenes represent a greater danger than assumed until now. Furthermore, this study provided evidence for induction of proinflammatory cytokines in the lungs, indicating that this organ is also a target organ for DON effects. Presence of other factors in grain, for example endotoxin or β-glucans could have a synergistic effect with DON and exacerbate the effects. Studies into dose-effect and short term chronic exposure should elucidate the toxic potential and its synergies with other compounds further. (Amuzie, Harkema, Pestka 2008) Corps *et al.* (2010)

Table 3. Summary of primary articles concerning airborne mycotoxin exposure measurements

Article Title	Sampling Method	Extraction Method	Analytical Method	Limit Of Detection (Sampling)	Limit Of Detection (Analysis)	Location of measurements	Mycotoxins measured	Circumstances of measurement	Number of measurements	Remarks
Bloom *et al.*, 2007	Vacuum (dust)/ cotton swabs	methanol/dichloromethane	HPLC-MS / GC-MS	Unknown	0,2 pg	Buildings with dampness or water damage history	sterigmatocystin, satratoxin G/H, verrucarol, trichodermol	Dust samples/cultures of dust samples/building materials	78	Dust samples were divided into settled and airborne dust samples
Brasel *et al.*, 2005a	Culture of *Stachybotrys chartatum* in experimental air sampling apparatus	methanol	ELISA/HPLC	Particles of 0.4 um	0,1 ng/ml	Lab setting, experimental air sampling apparatus	Satratoxin G/H, Roridin A, Verrucarin A, Deoxynivalenol, T-2 Toxin, Verrucarol, Diacetylverrucarol, Neosolaniol, Altenuene, Sterigmatocystin	Experimental Air sampling apparatus, lab conditions	12, including controls	Apparatus was specially build, fungus grown on wet ceiling tiles
Brasel *et al.*, 2005b	High volume air sampler	Freezing, dissolving in water	ELISA	32 conidia per cubic metre	3.1 pg per cubic metre	Several rooms in 8 mold-contaminated buildings	Satratoxin G/H, Roridin A, Verrucarin A	Indoor air samples, analysis of toxins by specific ELISA	99, across 8 buildings and 16 rooms	-
Bunger *et al.*, 2004	Spores, collected in composting plants, cultured on YES-agar	methanol/dichloromethane	HPLC-MS	Unknown	Unknown	Composting plants	Verruculogen, Fumagillin, Sterigmatocystin, Penitrem A, Roquefortine C	Fungi grown on composting plants, cultivated, then removed with methanol from agar culture dishes	Not specified	This paper was more cytotoxicity testing on cell lines (off topic)
Fischer *et al.*, 2006	Culturing, conidia removed	Not specified	HPLC-DAD	N.A.	N.A.	Lab setting, culturing	Aflatoxin, citrinin, cyclopenin, cyclopenol, mycophenolic acid, ochratoxin, patulin, penicillic acid, penitrem A, roquefortine C, sterigmatocystin, viridicatin	Mycotoxin measurements as indication of fungal species only, very limited measurements	Not specified	Paper aims only to identify fungal species by mycotoxin (off topic)

Table 3. Continued.

Article Title	Sampling Method	Extraction Method	Analytical Method	Limit Of Detection (Sampling)	Limit Of Detection (Analysis)	Location of measurements	Mycotoxins measured	Circumstances of measurement	Number of measurements	Remarks
Sawane *et al.*, 2005	High volume air sampler, 5 min samples in triplicate at each site	Agar plate pieces cut out, wetted with chloroform/methanol	Thin Layer Chromatography	N.A.	No quantification of mycotoxins performed, thus no LOD, only qualitative results	Houses and shops on 4 market locations in India, all poorly ventilated buildings	Roquefortin C, citrinin, rubratoxin B, cyclopiazonic acid, verucosidin, mitorubrinic acid + two unknown metabolites	Air samples were incubated on growth medium, and subcultured. Pieces of agar were cut out, and tested for mycotoxins by Thin Layer Chromatography.	Unclear, 384 air samples taken, but not all were tested for mycotoxins	Testing for mycotoxins was only a sidestudy in this paper, data are limited
Tangni *et al.*, 2007	Settled dust samples collected on filters sieved and homogenized	Methanol/water (50:50)	HPLC-UV	Dust collected 'sampled' by grain workers, method unknown	Citreoviridin: 0.01, citrinin: 0.0004, cyclopiazonic acid: 0.03, deoxynivalenol: 0.03, gliotoxin: 0.1, helvolic acid: 0.1, mycophenolic acid: 0.03, nivalenol: 0.05, ochratoxin A: 0.0001, patulin: 0.12, penicillic acid: 0.03, secalonic acid D: 0.01, sterigmatocystion: 0.1, zearalenol: 0.05, zearalenone: 0.015. All in ug/g	14 grain farmers and grain storage companies	Citreoviridin, citrinin, cyclopiazonic acid, deoxynivalenol, gliotoxin, helvolic acid, mycophenolic acid, nivalenol, ochratoxin A, patulin, penicillic acid, secalonic acid D, sterigmatocystion, zearalenol, zearalenone.	Samples were sieved, then homogenized. 2 extractions followed with acetonitril:water (90:10). Methanol:water (50:50) was used in the HPLC analyses.	14, tested for every mycotoxin mentioned	-
Wang *et al.*, 2008	Air sampler, pure water used to collect airborne dust	Methanol	HPLC	Unknown, but air sampler had flow rate of 12.5L/min, operated for 30 min Total: 375 L	0.02 ng/ml for Aflatoxin B1,B2,G1,G2, 0.05 ng/ml for ochratoxin, 0.4 ng/ml for zearalenone	Poultry house in Dalian, China	Aflatoxin B1, B2, G1, G2, Ochratoxin, Zearalenone	Samples taken in poultry house, tested for mycotoxins, 20 of 52 positive	52, of which 20 were positive for four toxins	-

Table 3. Continued.

Article Title	Sampling Method	Extraction Method	Analytical Method	Limit Of Detection (Sampling)	Limit Of Detection (Analysis)	Location of measurements	Mycotoxins measured	Circumstances of measurement	Number of measurements	Remarks
Halstensen *et al.*, 2006a	Settled dust, collected by paper filter cassettes, Airborne dust by vacuum	Acetonitrile:water (84:16)	GC-MS	2 L/min, 10-60 min breath samplers	T-2 toxin: 50-100ug/kg, HT-2 toxin: 30 ug/kg, DON: 20 ug/kg	Grain farms in Norway	T-2 toxin, HT-2 toxin, Deoxynivalenol	Samples of settled and airborne grain dust on grain farms in Norway	Settled: 109, Airborne dust: 112	Paper aims to predict trichothecene levels in grain dust by quantitative measurements of fungal DNA
Halstensen *et al.*, 2006b	Settled dust collected by vacuum, using paper filter cassettes	Acetonitrile:water (84:16)	GC-MS		T-2 toxin: 50-100ug/kg, HT-2 toxin: 30 ug/kg, DON, 3-acetylDON, 3,15 diacetoxyscirpenol: 20 ug/kg, Nivalenol: 50 ug/kg, Fusarenon-X: 40 ug/kg, monoacetoxyscirpenol: 10 ug/kg	Grain farm equipment on Norwegian grain farms	T-2 toxin, Nivalenol, fusarenon-X, HT-2, Deoxynivalenol, 3 acetyldeoxynivalenol, 4,15-diacetoxyscirpenol, monoacetoxyscirpenol	Samples of settled grain dust on Norwegian farms	109	
Moularat *et al.*, 2008	Aerosolization system, filters: PTFE; flow rate: 5L/min	Different mixtures tested, water/methanol mixture chosen	HPLC-UV	N.A.	8.5 ug/L	Lab setting, experimental air sampling/aerosolisation apparatus	Sterigmatocystin, Deoxynivalenol, Ochratoxine A	Lab setting: apparatus creates myctoxin aerosol, filters aerosol through PTFE filtersystem	4 growth substrates, each 3 mycotoxins, total n = 12	As stated in title, paper compares different methods to measure airborne mycotoxins and thus directly determine their rate of production
Panaccione *et al.*, 2005	Fungi from deceased parakeet and passive air sample (opening petri dish with growth medium) from a house	Methanol/water (80:20)	HPLC	N.A., extracts of grown conidia were directly analyzed by HPLC	Unknown	Detection of mycotoxins from cultured fungi isolates	Fumigaclavine C, festuclavine, fumigaclavine A, fumigaclavine B	Conidia of cultured fungi were removed, mycotoxins were extracted by methanol and measured by HPLC-MS	32, 8 media for 4 mycotoxins	Article measures mycotoxins in solutions of conidia extracts as an indicator of respirable mycotoxin concentration

researched roridin A (RA) responses in mice by exposing them to aerosolized RA; they assessed the persistence of the effects in repeated exposure to RA. They found several dose-dependent effects, including neutrophilic rhinitis with mucus hypersecretion, atrophy and exfoliation of nasal transitional and respiratory epithelium; repeated exposure to macrocyclic trichothecenes like RA might cause more severe and persistent effects than single acute exposures. The atrophy of the olfactory bulb also raised concern; the loss of smell associated with this effect could seriously impact human health. Several new neurocognitive symptoms have also been associated with heavy mold exposure by several animal studies, epidemiological studies and case studies. (Curtis and others 2004)

There is an accumulated weight of evidence associating indoor mold contamination to multisystem human adverse health effects. (Curtis and others 2004) The results suggest mycotoxin uptake and health problems of people living or working in buildings with *S. chartarum* are related and that these mycotoxins pose a serious health risk. (Straus and Wilson 2006)

Discussion

In general, there are many difficulties in making a risk assessment for airborne exposures to mycotoxins. A detailed risk assessment method is needed; just the enormous variety of the different mycotoxins is cause for further study into their (toxic) properties. More data about these metabolites will support more detailed risk assessment.

Many methods are available to researchers in the investigation of mycotoxins and their health risks; however, the different studies reviewed indicate that even the most utilized method, HPLC, still has its shortcomings. Standardization in measuring methods is also needed to increase reproducibility and comparability between studies. There are quite a few studies analyzing fungal species by PCR, microscopy and genetic analysis while measuring mycotoxins in all kinds of environments but there are few studies correlating these data.

The risk assessment for airborne mycotoxin exposure is complicated; many factors play a role in determining health risks; several toxicological and environmental studies have been performed, mainly in the last decade, but there are many uncertainties in the results of these studies. There are indications that airborne exposure to mycotoxins is more hazardous than oral exposure. A study using mice by Amuzie *et al.* (2008), covered earlier in this report found that DON evoked more toxicity and was more widely distributed through the tissues in mice exposed nasally in contrast to mice exposed to DON orally; furthermore, they reported that several other studies they examined came to identical conclusions. (Amuzie, Harkema, Pestka 2008)

The sources of exposure are another complication: not all sources have been equally researched; for example, smoking is not often considered as a source of mycotoxin exposure. Still, as Pauly *et al.* (2010) suggested, smoking can

indeed be a source of exposure to mycotoxins due to the production processes of tobacco. (Pauly and others 2010)

The number of different mycotoxins is another complicating factor for research; several kinds of mycotoxins have been more intensely studied than others. The trichothecenes are well studied for example, while other mycotoxins have not received as much attention from the scientific community. Many uncertainties remain in the knowledge about disease causing properties of many mycotoxins and health concerns. Although it has been established that in the case of macrocyclic trichothecenes people in moldy buildings inhale them, the concentrations to which these people are exposed remain to be studied further. (Straus and Wilson 2006)

There are more reasons besides the pure scientific ones, why more research into airborne exposure is needed; in the US alone, insurance companies spend many millions of dollars in lawsuits every year in cases related to moldy buildings. (Thacker 2004) Furthermore, since regulation is minimal, and mostly targeted at foodborne exposure, more research could also help to set maximum allowed concentrations with more precision.

Table 4. Summary of primary papers using rat & mouse experiments as models for airborne mycotoxin exposure

Reference	Extraction Method	Analytical Method	Limit Of Detection (Sampling)	Limit Of Detection (Analysis)	Mycotoxins measured	Circumstances of measurement	Number of measurements	Remarks
Alvarez-Garcia *et al.*, 2010	-	ELISA	-	-	Ribotoxin Asp f 1, α Sarcin	Inflammatory Airway Responses	6 groups of 5 mice	-
Amuzie *et al.*, 2008	Tissue extraction	PCR, ELISA	-	-	Deoxynivalenol	Oral/Nasal exposure, inflammatory response measurement	Several groups of mice n>4	Paper compared inflammatory response differences between nasal and oral mycotoxin exposure
Corps *et al.*, 2010	Tissue extraction	PCR, immunohistochemistry, Morphometric analysis, cytology, flow cytometry	N.A.	N.A.	Roridin A	Respiratory airway responses of mice	6 groups of 6 mice	Only one Mycotoxin tested, paper did not aim to test exposure

Conclusion

Airborne exposure is, compared to foodborne exposure, relatively little researched; therefore, little 'hard' data is available and risk assessment is therefore difficult. Although feed/foodstuffs have maximum allowed levels of mycotoxins, there are currently no maximum airborne levels of mycotoxins. (Wang and others 2008) However, for chemical intake, the Threshold of Toxicological Concern (TTC) was established as a guideline; this TTC applies mainly to oral and dermal exposure. The TTC was intended to estimate health risks of a certain substance without prior testing or chemical-specific toxicity data with exposure over a 70-year lifespan. The airborne equivalent was recently established; this Concentration of No Toxicological Concern (CoNTC), set at 30 ng/m^3, represents a very generic airborne concentration limit. Hardin *et al.* (2009) already concluded that in some agricultural conditions, concentrations of mycotoxins in the air exceed the CoNTC; however, they also found that in indoor environments, the levels seem to remain below the CoNTC threshold. (Hardin and others 2009)

The sources of mycotoxins are in principle quite clear: the fungi that produce mycotoxins are the sources. A variable in the sources is the production of mycotoxins; fungi do not always produce mycotoxins, and many can also produce different toxins. So a certain amount of fungi does not always produce the same toxin in the same quantities.

The concentration in air is influenced by several of the same factors as the sources of mycotoxins mentioned above; however, although several methods are available and many more are being investigated, airborne concentration data remains scarce. Uncertainties in methods and setup of the different studies performing these methods also complicate providing airborne concentrations. The limits of detection also play a role in the differences in concentration measurements; furthermore, since there are many different kinds of mycotoxins, with very different (chemical) properties, one method often measures only a few types of mycotoxins.

The exposure measurements encounter the same problems, but further complicated by lacks of data about the interaction between the body and the mycotoxins to which it is exposed. The murine models described above are a good start in elucidation of these interactions. In some settings, the concentration is much higher and therefore the exposure is much higher than in other settings. Grain farms, subject of several studies into mycotoxin exposure, have been reported to have very high airborne mycotoxin concentrations, while normal, undamaged buildings should have a very low airborne concentration of mycotoxins.

The dose of toxin to reach the target organ is also very difficult to establish: since there is a large variety in toxins there is also a large variety in target organs. While aflatoxin might cause liver damage, the trichothecenes mainly

cause immunity related injury, for example. Furthermore, the difference in other properties of mycotoxins also complicates matters; again, the murine models help to clear up questions but many still remain.
The health effects of mycotoxins are very diverse so the different mycotoxins have different target organs, and consequently cause different health effects. These can range from relatively mild ones like vomiting (Deoxynivalenol) to liver cancers (aflatoxin B1).
In general, the risk of airborne exposure to mycotoxins is difficult to quantify, since much research is still needed in all aspects of risk assessment. Several methods are available to make an estimate, such as animal models; but while the last decade saw a substantial increase in research into airborne exposure to mycotoxins, much work still needs to be done. Studies into the toxicological part of risk assessment are needed as well as exposure studies; furthermore, research needs to be more uniform in setup, as this would greatly improve comparability between studies. Meanwhile, precautions should be taken in dealing with places that have a higher risk of having higher concentrations of mycotoxins in the air, like grain farms or water-damaged buildings.

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