

CPSC Staff Statement on Toxicology Excellence for Risk Assessment (TERA) Report “Review of the Health Risks of Mold, Health Effects of Molds and Mycotoxins”¹

July 2015

The report titled, “Review of the Health Risks of Mold, Health Effects of Molds and Mycotoxins,” presents basic mold characteristics and was performed by TERA under Contract CPSC-D-12-0001, Task Order 0013. A second report, “Review of the Health Risk of Mold, Basic Mold Characteristics,” can be found under a separate cover. Consumer exposure to mold on a product may be more frequent and direct than exposures that might occur in a building setting, making remediation even more important for products with mold contamination. Therefore, this contract was initiated for staff to gain a better understanding of these hazards and the new information that has been developed over the past several years on mold characteristics and toxicity.

The available data on health effects associated with mold in each genus are reviewed in the first section. For each genus, the uptake and system spread and health effects in humans and/or animals are discussed. Following that is a discussion of the health effects associated with key mycotoxins produced by the genera of interest. The physical and chemical characteristics, toxicokinetics, and animal and human hazard information are included in these discussions.

Historically, much of the documented human exposure to molds and mycotoxins has been via food. This means that most of the available experimental data for mycotoxins are for the oral route, often in the context of dietary exposure; parenteral dosing studies are also often available. Generally speaking the data available for the inhalation and dermal routes are very limited. In contrast to the mycotoxins, inhalation and dermal exposure to the mold (*e.g.*, spores) may also occur.

In this report, Table 1 summarizes the key information on the basic mold characteristics (see the Basic Mold Characteristics report for more details), together with the health effects associated with each genus. The summary of the toxin effects (irritation, kidney, liver, developmental, cancer) is based primarily on animal data, and human data were used when available. Information on irritant effects is from testing of the mycotoxins in animals, or reports from human exposure to the mold or mycotoxins; controlled animal testing for irritancy was not done for the molds themselves.

¹ This statement was prepared by the CPSC staff, and the attached report was produced by TERA for CPSC staff. The statement and report have not been reviewed or approved by, and do not necessarily represent the views of, the Commission.

Health effects associated with these different molds in humans include: allergic reactions, sensitization, asthma, neurotoxicity, sinusitis, otomycosis, onychomycosis, keratitis, respiratory infections, skin infections, and systemic infections.

Determining which mycotoxins are produced by which genus is difficult. This reflects differences in toxin production by different strains or species within a genus and data gaps, or the tendency of review articles to focus on primary toxins and classes of toxins, rather than identifying each toxin (or each major toxin) produced by a genus. Health effects associated with mycotoxins in humans include: hepatic toxicity, cancers (liver, esophageal, lymphoma, skin, and gastric), nephrotoxicity, hypertension, hyperlipidemia, immunosuppression, and nasal irritation.

Review of the Health Risks of Mold

Completed Under Contract CPSC-D-12-0001

Task Order 0013

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Health Effects of Molds and Mycotoxins

Final Report, July 13, 2015

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3 Effects of the Mold as an Organism

3.1 Introduction

This volume reviews the key health effects associated with the molds and mycotoxins addressed in this report. The available data on health effects associated with the mold of each genus is reviewed in the first section. Following that is a discussion of the health effects associated with key mycotoxins produced by the genera of interest. The approach utilized for this report is based on a weight of evidence understanding of the relevant effects and should not be considered exhaustive. The focus of the review is on a weight of evidence understanding of the relevant effects and associated effect levels. There was no attempt to conduct an exhaustive review of the literature, or to capture every effect that has been attributed to a mold or mycotoxin. To that end, most of the data were extracted from authoritative reviews, wherever possible. In particular, several of the key reviews on mycotoxins were developed by the European Food Safety Authority (EFSA) and the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Other key reviews were from the U.S. Environmental Protection Agency (EPA) and the National Toxicology Program (NTP). Key studies were also reviewed for each of the mycotoxins.

Historically, much of the documented human exposure to molds and mycotoxins has been via food. This means that most of the available experimental data for mycotoxins are for the oral route, often in the context of dietary exposure; parenteral dosing studies are also often available. Generally speaking the data available for the inhalation and dermal routes are very limited.

Some limited information on effects of inhaled mycotoxins was provided by EPA (2004), but the mold and/or mycotoxin to which people were exposed was often not clear. Toxicity via the oral route is expected to be much higher than that via the dermal route (due to low dermal absorption).

In contrast to the mycotoxins, inhalation and dermal exposure to the mold (e.g., spores) may occur in a consumer context. There are typically very few controlled studies of exposure of experimental animals to the organisms, and many of the available studies were via non-environmentally relevant exposure routes. Thus, health effect data for the molds is primarily from human studies, predominantly case reports, with a few formal epidemiology studies. For these data, it is known that there was exposure to mold, but data on the specific genus are mixed. In some cases, the genus is identified based on either air monitoring (e.g., for people exposed to mold in buildings) or analysis of the source (e.g., contaminated grain). However, in many cases, exposures were to multiple genera, and it is often difficult or impossible to identify which mold genus (or which genera) was associated with observed effects. Even when more controlled analyses are conducted (e.g., evaluation of the correlation of asthma with positive skin-prick tests), definitive results are often precluded by cross-reactivity among genera, as discussed in the context of individual genera. The exposure route is typically not identified but, based on the

exposure scenarios, exposure would be expected to be predominantly via the inhalation and dermal routes. Thus, these exposures, although not well characterized, may better reflect exposures seen with consumer products than would oral studies. However, dose-response information is lacking for almost all of the organisms.

Several additional considerations are worthy of note. It is recognized that real-world exposures do not generally occur to mycotoxins in isolation. In particular, several genera, such as *Aspergillus* and *Penicillium*, produce multiple mycotoxins. (See Table 3 in Section 4.) The health effects observed in real world contexts may reflect the interactions among multiple mycotoxins, or even the mold and mycotoxins. Such interactions have not been considered in this report.

The broader complexities of sick building syndrome are also beyond the scope of this report. The asthma and allergic rhinitis associated with mold exposure is documented in the context of individual genera. However, EPA (1994) notes that “many patients and some physicians have attributed cognitive and other neurological syndromes to mold exposures. There is no consensus as to the nature, pathophysiology, or etiology of these syndromes.” This report does, however, discuss the controversy regarding health effects associated with *Stachybotrys*, or “black mold” exposure.

Table 1 summarizes the key information on the basic mold characteristics (see Volume 1 for more details), together with the health effects associated with each genus. For completeness, minor toxins are noted, but these were not evaluated in detail and so are not included by name in Table 1. (See Section 4 for more information.) To aid in evaluating the relationship between reported symptoms and specific exposures, Table 2 summarizes some of the key categories of health effects that are associated with each genus. The summary of the toxin effects (irritation, kidney, liver, developmental, cancer) is based primarily on animal data, and human data were utilized when available. Note that information on irritant effects is from testing of the mycotoxins in animals, or reports from human exposure to the mold or mycotoxins; controlled animal testing for irritancy was not done for the molds themselves. Note that the observation of an effect in an experimental animal species does not necessarily mean that the same target will be affected in humans.

Table 1. Summary of Key Mold Characteristics and Health Effects

Genus/Class	Visual Appearance	Growth Characteristics	Expected Growth Location	Toxin Production	Health Effects/Targets Associated with Toxin	Health Effects Associated with Mold	
						Site of Contact/ Allergy/Asthma	Systemic Effect
Alternaria	Dark colored spores	Seasonal increase related to rainfall and temperatures (Increase Jun-Oct)	Ubiquitous	Alternariol and related Alttoxins Tetramic acids	Limited data; parenteral data suggest may have development effects; mutagen	Opportunistic infections primarily of skin, eye and nose. Allergen and asthmagen	Opportunistic pathogen
Aspergillus	Powdery white, green, yellowish, brown or black colonies	Fast growing Thermotolerant	Ubiquitous; cellulose, water-damaged buildings	Aflatoxin (1) Citrinin (2) Ochratoxin (3) Sterigmatocystin (4) Various other toxins	(1) Liver, immune, cancer; limited data suggest skin and eye irritant (2) Kidney, liver; nasal irritant (limited data), eye irritant (3) Kidney, liver, immune, neuro, repro, developmental, cancer (4) Liver, kidney, cancer	Superficial infection of nose, skin, ears, nails; sinusitis; aspergillosis. Allergen and asthmagen	Opportunistic invasive infections
Chaetomium	Brown with "hairs"	Prefers neutral pH; grows at pH 4.3 to 9.4. Acidic environment favors sporulation	Affinity to cellulose; water-damaged buildings	Chetoglobosins Many minor toxins	Limited data: Possible liver, immune, and developmental effects, but data inadequate.	Infection of skin, nails, and cornea – usually opportunistic	Opportunistic pathogen

Genus/Class	Visual Appearance	Growth Characteristics	Expected Growth Location	Toxin Production	Health Effects/Targets Associated with Toxin	Health Effects Associated with Mold	
						Site of Contact/ Allergy/Asthma	Systemic Effect
Cladosporium	Pigmented olive-green to black. Velvet to powdery.	Hypersaline environments, Low water activity, limited growth above 35°C	Affinity to cellulose, water-damaged buildings <i>Not</i> on concrete, glass or plastics	None	No toxins	Infection of skin, nails, and cornea. Allergen and asthmagen	Opportuniistic pathogen
Dicyma	Varies. White to grey-greenish to black	Grows well in dark	Organic materials	Minor toxins	No data	Maxillary Sinusitis	No data
Epicoccum	Red or orange pigments (inhibited by intense light)	Acidic environment	Fruit and Vegetables; Clay materials; Quartz	None	No toxins	Infection of skin and lung. Suggested allergen and asthmagen	No data
Malassezia	Creamy, white with brittle texture	Lypophilic, Most lipid dependent	Common on skin (healthy and diseased)	None	No toxins	Pityriasis versicolor – hyperpigmentation; psoriasis, seborrheic dermatitis, dandruff, eczema, nail infection. Allergen and asthmagen	Opportunistic

Genus/Class	Visual Appearance	Growth Characteristics	Expected Growth Location	Toxin Production	Health Effects/Targets Associated with Toxin	Health Effects Associated with Mold	
						Site of Contact/ Allergy/Asthma	Systemic Effect
Penicillium	White – green fluffy masses; Varies with temperature , pH and humidity	Rapid growth, Musty odor due to VOCs	Food; Affinity to cellulose, water-damaged buildings <i>Not</i> on concrete, glass or plastics	Citrinin (1) Ochratoxin (2)	(1) Kidney, liver; ; nasal irritant (limited data), eye irritant (2) Kidney, liver, immune, neurological, reproductive, developmental, cancer	Superficial infection of nose, skin, ears, nails; Allergen and asthmagen;	Opportunistic
Phoma	Flat white to grey with brown pigment	Rapid growth, Conidia are enclosed so not ubiquitous airborne organism	Cruciferous vegetables, cabbages, mustard, Asbestos, cement, oil-paint, plaster, crockery	Various minor toxins	No data	Superficial and invasive infections of skin, nails and lung. Allergen and asthmagen. Associated with eye, skin, respiratory tract irritation but not causally linked	Opportunistic – limited data

Genus/Class	Visual Appearance	Growth Characteristics	Expected Growth Location	Toxin Production	Health Effects/Targets Associated with Toxin	Health Effects Associated with Mold	
						Site of Contact/ Allergy/Asthma	Systemic Effect
Stachybotrys	Black	Requires moisture but grows well in most temperatures. Spores can survive winter and remain viable for decades.	Affinity to cellulose, maybe present on concrete, glass or plastics.	Cyclosporine (1) Trichothecenes, including satratoxins (2) Various other toxins VOCs include alcohols, ketones, hydrocarbons, ethers and esters	(1) Immune, kidney, liver, cancer (2) Respiratory	No infections, all effects due to toxins; Eye skin and respiratory irritation reported but causality not shown No clear association with allergy/asthma;	Effects are due to the mycotoxins
Rhizopus (Zygomycetes)	Grey-white colonies that are cottony or wooly	Thermotolerant, Rapid Growth	Ubiquitous on organic surfaces	None	No toxins	Infections of nose, lung, skin, gastrointestinal tract	Opportunistic, but potentially severe

Genus	Allergy or Asthma	Irritation	Infection	Kidney	Liver	Developmental	Cancer
Alternaria	Yes	No data	Opportunistic	Inadequate data	Inadequate data	Limited data, but possible based on parenteral data	Inadequate data
Aspergillus	Yes	<i>Nasal</i> , eye, skin	Opportunistic	Yes	Yes	Yes, but may be secondary to maternal toxicity	Yes
Chaetomium	No	No data	Opportunistic	Inadequate data	Possible, but inadequate data	Possible, but inadequate data	Inadequate data
Cladosporium	Yes	No data	Opportunistic	No toxins	No toxins	No toxins	No toxins
Dicyma	No data	No data	Opportunistic	No data	No data	No data	No data
Epicoccum	Suggested	No data	Opportunistic	No toxins	No toxins	No toxins	No toxins
Malassezia	Yes	No data	Opportunistic	No toxins	No toxins	No toxins	No toxins
Penicillium	Yes	<i>Nasal</i> , eye	Opportunistic	Yes	Yes	Yes, but may be secondary to maternal toxicity	Yes
Phoma	Yes	Associated with eye, skin, respiratory tract irritation but not causally linked	Opportunistic	Inadequate data	Inadequate data	Inadequate data	Inadequate data
Stachybotrys	No clear evidence	Eye skin and respiratory irritation reported but causality not shown	No	Yes	Yes	At maternal toxic dose	Yes
Rhizopus	No	No data	Opportunistic	No toxins	No toxins	No toxins	No toxins

(Zygomycetes)			c				
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Table 2. Summary of Key Health Effects by Organism, Based on Animal and Human Data¹

¹Bolded entries are based on human data, while the bolded and italicized entry means that the human data support effects seen in animal studies

3.2 Alternaria

3.2.1 Uptake and Systemic Spread

Alternaria, a spore-producing fungus, is normally present on the skin and is an opportunistic pathogen. *Alternaria* can also be found on the conjunctiva (the moist membranes on the inner surface of the eyelids). The routes of entry are by inhalation, dermally (through breaks in the skin), and ocularly, after corneal trauma. *Alternaria* infections are more prevalent in patients with immunosuppression (Pastor and Guarro, 2008). *Alternaria* spores are 20-200 µm in length and 7-18 µm in width. This means that inhaled spores will be deposited primarily in the nose and mouth, although some may reach the upper respiratory tract (EFSA, 2008).

3.2.2 Health Effects in Humans

3.2.2.1 Site of Contact Effects

Alternaria alternata and *A. tenuissima* are the most frequent species found in human infection, although six other species have been implicated (DeLuca, 2007). The incidence of *Alternaria* infections in onychomycosis (fungal infections of the nails) was very low (<2.5%), with most caused by *A. alternata*. The most frequent and common *Alternaria* infections are infections of the skin, with approximately 90% being cutaneous infections and characterized by erythema, desquamation of the skin, red papules and ulceration. Approximately 30% of the patients with cutaneous infections were on immunosuppressive treatment (Pastor and Guarro, 2008). Subcutaneous skin infections are also common.

Oculomycosis (fungal infections of the eye), onychomycosis and invasive and non-invasive rhinosinusitis (long-term nasal congestion and thick mucus secretions) are other *Alternaria* effects reported. Contact with the soil and/or garbage are common exposure scenarios in cases of oculomycosis and onychomycosis (Pastor and Guarro, 2008). For ocular infections, the incidence of *Alternaria* ranges from 3.3 to 10.4%. Many of these cases were related to ocular trauma and exposure to soil and/or garbage (Pastor and Guarro, 2008).

Chronic rhinosinusitis is estimated to occur in 14% of the US population, and exposure to airborne *Alternaria* is a major suspected cause (Ponikau et al., 2005). The most common fungi isolated from sinusitis are *Aspergillus* but *Alternaria* is commonly associated with this disease as well (Shin et al., 2004). Immunosuppression does not appear to be a risk factor in chronic rhinosinusitis (Pastor and Guarro, 2008). Chronic rhinosinusitis patients have a specific immune response to fungi. The mean serum IgG levels specific for *Alternaria* were fivefold higher in chronic rhinosinusitis patients as compared to healthy patients (Ponikau et al., 2005) with no history of allergic diseases, asthma, or chronic rhinosinusitis (Shin et al., 2004).

3.2.2.2 Irritation

No information was located on the potential for *Alternaria* to cause irritation.

3.2.2.3 Sensitization, Inflammation, Allergy and Asthma

Alternaria was found in nearly all patients with chronic rhinosinusitis (Lanza, 2006). Shin et al. (2004) reported that patients with chronic rhinosinusitis had an exaggerated allergic response to *Alternaria*, suggesting sensitization. Type I allergies (allergies that are antibody-mediated) can be caused by *Alternaria alternata* and *A. tenuis* (Heibling and Reimers, 2003). Children and young adults have a high risk of allergic respiratory condition if they have skin reactions to the antigens of *A. alternata* (Martinez-Canavate et al., 2007) or if they are exposed to high (>1000 spores/m³)² atmospheric spore counts (Heibling and Reimers, 2003). The respiratory conditions present themselves as severe asthma (Heibling and Reimers, 2003). Approximately 10 % of patients with respiratory allergies had been sensitized to *Alternaria* and/or *Cladosporium* (Martinez-Canavate et al., 2007). D'Amato (1997) reported that up to a 20% prevalence of fungal sensitization was found in Spain (Martinez-Canavate Burgos, 2007), whereas, in the US and Scandinavia, the prevalence of fungal sensitization was only 3-4% (De Luca, 2007).

Asthma severity is strongly linked to *Alternaria* sensitivity. Airway hyper-responsiveness, the exaggerated narrowing of the airways after the inhalation of allergenic stimuli, is a key feature of asthma. In the United States, 80% of individuals with confirmed asthma have positive allergic reaction to *Alternaria* (Nasser and Pulimood, 2009). In one study, 38 % of 12,086 asthmatic children residing in US inner cities had positive skin tests to *Alternaria*. In school aged children, sensitization to *Alternaria* correlated with asthma (Perzanowski et al., 1998). Thunderstorm induced asthma is increasing and also has been associated with sensitization to *Alternaria* spores (Nasser and Pullimood, 2009). Allergic bronchopulmonary mycosis caused by *Alternaria* has been reported (Singh and Denning, 2012).

3.2.2.4 Systemic Effects

Signs of neurotoxicity (weakness and numbness in legs, dizziness, loss of memory, light-headedness, vertigo, fatigue) were observed in 12 female office workers chronically exposed to a toxicogenic mold including *Alternaria tenuis* (EFSA, 2008). These effects can not be definitively associated with the *Alternaria* exposure, in light of the mixed exposure and subjective nature of the symptoms.

Systemic infections with *Alternaria* are rare and found primarily in immunosuppressed people. For example, phaeohyphomycosis (presenting as a deep subcutaneous fungal infection caused by *Alternaria*) was observed in a renal transplant patient (Salido-Vallejo 2014). Invasive *A.*

² It is not clear from the text whether this number refers to total spores or *Alternaria* spores.

alternata infections were reported in a patient receiving a bone marrow transplant (Ferreira et al., 2013) and in a cardiac transplant recipient (Cascio et al., 2004).

3.2.2.5 Health Effects in Animals

Dogs and cats can have skin infections caused by *Alternaria* (Bernardo et al., 2005). In immune compromised cats and dogs, the *Alternaria* infection can become a systemic infection (Dedola et al., 2010). The presence of scaling on the skin of dogs and cats plus the possibility of the aerosolization of *Alternaria* spores can increase the frequency and intensity of an asthmatic attack in patients already sensitized to *Alternaria* (Singh and Denning, 2012; Jang et al., 2007; Salo et al., 2006).

3.2.3 References

Bernardo, F., Lança, A., Guerra, M. M., Marina Martins, H., 2005. Dermatophytes isolated from pet, dogs and cats, in Lisbon, Portugal (2000-2004). RPCV (Revista Portuguesa de Ciencias Veterinarias). 100, 85-88.

Cascio, G. L., Ligozzi, M., Maccacaro, L., Fontana, R., 2004. Utility of molecular identification in opportunistic Mycotic infections: A case of cutaneous *Alternaria infectoria* infection in a cardiac transplant recipient. J Clin Microbiol 42, 5334–5336

D'Amato, G., Chatzigeorgiou, G., Corsico, R., Gioulekas, D., Jäger, L., Jäger, S., Kontou-Fili, K., Kouridakis, S., Liccardi, G., Meriggi, A., Palma-Carlos, A., Palma-Carlos, M. L., Pagan Aleman, A., Parmiani, S., Puccinelli, P., Russo, M., Spieksma, F., Torricelli, R., Wüthrich, B., 1997. Evaluation of the prevalence of skin prick test positivity to *Alternaria* and *Cladosporium* in patients with suspected respiratory allergy. A European multicenter study promoted by the Subcommittee on Aerobiology and Environmental Aspects of Inhalant Allergens of the European Academy of Allergology and Clinical Immunology. Allergy. 52, 711-716. (Abstract only)

Dedola, C., Stuart A.P., Ridyard, A.E., Else, R.W., van den Broek, A.H., Choi, J.S., de Hoog, G.S. Thoday, K.L. 2010. Cutaneous *Alternaria infectoria* infection in a dog in association with therapeutic immunosuppression for the management of immune-mediated haemolytic anaemia. Vet Dermatol. 21(6), 626-34.

De Lucca, A. J., 2007. Harmful fungi in both Agriculture and Medicine. Rev Iberoam Micol. 24, 3-13.

de Sousa Ferreira, I., Teixeira, G., Abecasis, M., 2013. *Alternaria alternata* invasive fungal infection in a patient with Fanconi's Anemia after an unrelated bone marrow transplant. Clin Drug Investig. 33 S33–S36.

- EFSA (European Food Safety Authority). 2008. Scientific information on mycotoxins and natural plant toxicants. CFP/EFSA/CONTAM/2008/01. European Food Safety Authority.
- Grael, C., Tapias, G., Valencia, M., Randazzo, L., Anglada, E., Olivé, A., 1993. Allergy to *Alternaria*. I. Clinical aspects. *Allergol Immunopathol (Madr)*. 21, 15-19. (Abstract only)
- Helbling, A., Reimers, A., 2003. Immunotherapy in fungal allergy. *Curr Allergy Asthma Rep*. 3, 447-453.
- Jang, K.-S., Yun, Y.-H., Yoo, H.-D., Kim S H, 2007. Molds isolated from pet dogs. *Mycobiology*. 35, 100-102.
- Lanza, D. C., Dhong, H. J., Tantilipikorn, P., Tanabodee, J., Nadel, D. M., Kennedy, D. W., 2006. Fungus and chronic rhinosinusitis: from bench to clinical understanding. *Ann Otol Rhinol Laryngol Suppl*. 196, 27-34.
- Martínez-Cañavate Burgos, A., Valenzuela-Soria, A., Rojo-Hernández, A., 2007. Immunotherapy with *Alternaria alternata*: Present and future. *Allergol Immunopathol (Madr)* 35, 259-263.
- Nasser, S. M., Pulimood, T. B., 2009. Allergens and thunderstorm asthma. *Curr Allergy Asthma Rep*. 9, 384-390.
- Pastor, F. J., Guarro, J., 2008. *Alternaria* infections: laboratory diagnosis and relevant clinical features. *Clin Microbiol Infect*. 14, 734-746.
- Perzanowski, M. S., Sporik, R., Squillace, S. P., Gelber, L. E., Call, R., Carter, M., Platts-Mills, T. A., 1998. Association of sensitization to *Alternaria* allergens with asthma among school-age children. *J Allergy Clin Immunol*. 101, 626-632.
- Ponikau, J. U., Sherris, D. A., Kephart, G. M., Adolphson, C., Kita, H., 2005. The role of ubiquitous airborne fungi in chronic rhinosinusitis. *Curr Allergy Asthma Rep*. 5, 472-476.
- Salido-Vallejo, R., Linares-Sicilia, M. J., Garnacho-Saucedo, G., Sánchez-Frías, M., Solís-Cuesta, F., Gené, J., Moreno-Giménez, J. C., 2014. Subcutaneous phaeohyphomycosis due to *Alternaria infectoria* in a renal transplant patient: Surgical treatment with no long-term relapse. *Rev Iberoam Micol*. 31, 149-151.
- Salo, P. M., Arbes, S. J., Sever, M., Jaramillo, R., Cohn, R. D., London, S. J., Zeldin, D. C., 2006. Exposure to *Alternaria alternata* in US homes is associated with asthma symptoms. *J Allergy Clin Immunol*. 118, 892-898.
- Shin, S. H., Ponikau, J. U., Sherris, D. A., Congdon, D., Frigas, E., Homburger, H. A., Swanson, M. C., Gleich, G. J., Kita, H., 2004 Chronic rhinosinusitis: An enhanced immune response to ubiquitous airborne fungi. *J Allergy Clin Immunol*. Dec. 114, 1369-1375.

Singh, B., Denning, W. D., 2012. Allergic bronchopulmonary mycosis due to *Alternaria*: Case report and review. *Med Mycol Case Rep.* 1, 20–23.

3.3 Aspergillus

3.3.1 Uptake and Systemic Spread

Virulence depends on both a combination of biological factors, such as the structure of the mold and its ability to adapt to stress, and the immune status of the host (Binder and Lass-Flörl, 2013). *Aspergillus* adapts well to a broad range of environmental conditions, including heat, which makes it a successful pathogen. It also produces small airborne conidia, which are easily dispersed in the environment and respired (Binder and Lass-Flörl, 2013). Respired conidia are scrubbed from the airway via cilia in the respiratory epithelium (mucociliary clearance), but some conidia may still pass into the lung (Binder and Lass-Flörl, 2013). In healthy individuals, these fungal conidia are generally eliminated through phagocytic defenses, but infection of the lung is more likely to occur in persons with depressed immune systems (Binder and Lass-Flörl, 2013; Brakhage, 2005). Invasive infection occurs in the lung and sinus tissues after the mucosal surfaces are breached, resulting in tissue damage and, eventually, dissemination through the blood stream (Hope et al., 2005).

3.3.1.1 Health Effects in Humans

Of approximately 250 species of *Aspergillus*, 40 are associated with human disease (Kilch, 2009). Most *Aspergillus* infections are caused by *A. fumigatus*, *A. flavus*, *A. terreus*, *A. niger*, and *A. nidulans* (Binder and Lass-Flörl, 2013; De Lucca, 2007; Hope et al., 2005; Kilch, 2009), but *A. fumigatus* causes more infections than any other mold (Rizzetto and Cavalieri, 2011). These infections are classified into three categories: non-invasive infection (colonization of mucosal surfaces), invasive infection (the growth of fungi in tissues), and allergic or hypersensitivity diseases (Binder and Lass-Flörl, 2013).

3.3.1.2 Site of Contact Effects and Local Infection

Aspergillus colonizes the airways, nose, sinuses (sinusitis), skin, ear canals (otomycosis), and nails (onychomycosis) and causes persistent superficial infection without progressing to invasive infection and disease (Aznar et al., 1989; Binder and Lass-Flörl, 2013; De Lucca, 2007; Versalovic et al., 2011). *Aspergillus* can also cause corneal infections (keratitis) following ocular injury with subsequent contamination, particularly among agricultural workers (De Lucca, 2007). Other superficial infections include tracheobronchitis (Marques et al., 2000; Versalovic et al., 2011) and saprophytic bronchopulmonary aspergilloidosis, or aspergilloma (Versalovic et al., 2011). Aspergilloma (chronic mycetoma), a generally benign fungus ball (Binder and Lass-Flörl, 2013; Kilch, 2009), is generally found in the lung and is commonly found in people with pre-existing damage to the lung (e.g., tuberculosis infection or fungal sinusitis). Symptoms

include mild hemoptysis (coughing up bloody mucus), chronic cough, weight loss, and sometimes fever (Kilch, 2009). However, aspergillomas have also been reported in the sinus cavity and in immunocompetent people, although rarely (Binder and Lass-Flörl, 2013). Acute pulmonary aspergillosis has also been reported in healthy men after spreading contaminated bark chips (Kilch, 2009).

Invasive aspergillosis (IA) is an opportunistic mold infection that occurs primarily in the respiratory tract, particularly amongst immunocompromised persons (i.e., patients with bone marrow transplants, hematology malignancies, solid organ transplants, hematopoietic stem cell transplant recipients, human immunodeficiency virus [HIV]-infections, intense chemotherapy, and critically ill patients in intensive care units) and persons with chronic granulomatous disease, an inherited disorder in which immune cells are unable to kill bacteria and fungi (Ascioglu et al., 2002; Ben-Ami et al.; Binder and Lass-Flörl, 2013; Brakhage, 2005; De Lucca, 2007; De Pauw et al., 2008; GAO, 2008; Hope et al., 2005; Kilch, 2009; Versalovic et al., 2011). In persons with a weakened immune system, the inhaled *Aspergillus* conidia germinate and produce hyphae that invade pulmonary tissue (De Lucca, 2007). IA is a significant contributing factor to morbidity and mortality among immunocompromised patients (Binder and Lass-Flörl, 2013; Brakhage, 2005; Hope et al., 2005), and the mortality rate in infected immunocompromised patients is very high (> 90%) (De Lucca, 2007). *A. fumigatus* is the cause of about 90% of all pulmonary fungal infections (De Lucca, 2007). *A. flavus* is the second-most cause of IA and superficial infection (Hedayati et al., 2007). Infections caused by *A. terreus* are less common, but have a higher associated mortality rate, likely because it is resistant to amphotericin B (Kilch, 2009). Other risk factors include prolonged neutropenia (abnormally low levels of neutrophils in the blood), broad spectrum antibiotic treatment, severe immunosuppression, inherited immune defects, underlying diseases and conditions, biological factors (e.g., old age), environmental exposure (e.g., hospitalized patients), antifungal prophylaxis, and iron overload (elevated ferritin) (Binder and Lass-Flörl, 2013; Brakhage, 2005). The associative nature of some risk factors, such as corticosteroid treatment and infection with cytomegalovirus, are not agreed upon (Binder and Lass-Flörl, 2013; Brakhage, 2005). In one study, nine cases of fatal IA were linked to the presence of *A. terreus* in potted plants in hospital rooms (Lass-Flörl et al., 2000). Although it is less common, in HIV-infected patients, IA can occur outside of the respiratory tract, specifically in the central nervous system (Mylonakis et al., 2000; Shinn and Griffin, 2003).

Sino-orbital aspergillosis is another, usually fatal, progressive and opportunistic *Aspergillus* infection in immunocompromised (e.g., acquired immunodeficiency syndrome [AIDS]) patients (De Lucca, 2007; Marques et al., 2000). Aspergilli can also cause fungal rhinosinusitis, which can lead to invasive *Aspergillus* sinusitis, a fatal, but uncommon, disease (Binder and Lass-Flörl, 2013; Kilch, 2009).

Non-invasive colonization and allergy are generally the only diseases observed in immunocompetent persons (Brakhage and Liebmann, 2005). However, *Aspergillus* has been reported to cause chronic sphenoid sinusitis, or an infection of the sphenoid sinuses, in healthy individuals (De Lucca, 2007). These sphenoid infections can progress to intracranial IA, a largely fatal condition (De Lucca, 2007). At least one case of an IA brain lesion is reported in an immunocompetent patient suffering from chronic obstructive pulmonary disease (Kilch, 2009). *Aspergillus* is also associated with chronic cavitary pulmonary aspergillosis (CCPA), a disease where cavities are present in the lungs, but the onset is not associated with any overt disease or immune deficiency (Binder and Lass-Flörl, 2013; Kilch, 2009). A case of infective endocarditis on a native heart valve caused by *A. terreus* has also been reported in a person with no immunosuppression or heart disease (Kilch, 2009).

There is some limited evidence of an association between *Aspergillus* exposure and disease of the lower respiratory tract (e.g., bronchitis and pneumonia) in adults and children (GAO, 2008), especially in immunocompromised patients (Alangaden et al. 2002).

Overall, exposure to *Aspergillus* is associated with a number of non-invasive and invasive infections and effects, including superficial colonization, tracheobronchitis, aspergillosis, aspergilloma, IA, sinusitis, CCPA, endocarditis, bronchitis, and pneumonia.

3.3.1.3 Irritation

No information was located on the potential for *Aspergillus* to cause irritation.

3.3.1.4 Sensitization, Inflammation, Allergy, and Asthma

Aspergilli, specifically *A. fumigatus*, *A. Flavus*, and *A. niger*, cause a variety of allergic reactions, including rhinitis, conjunctivitis, coughing, and even pulmonary airway obstruction or anaphylaxis (Kilch et al., 2009). Specific allergic diseases associated with *Aspergillus* are allergic *Aspergillus* sinusitis, IgE-mediated asthma, allergic bronchopulmonary aspergillosis (ABPA), and hypersensitivity pneumonitis (De Lucca et al., 2007).

Most fungal allergens, including those isolated from multiple *Aspergillus* species, are located in germinating spores, hyphal tips, and mycelia (HUD, 2006), and are released by germination (Sporik et al., 1993). Environmental exposure to *Aspergillus* spores is less likely to be the cause of allergy than exposure to *Aspergillus* that has germinated in the respiratory tract (Sporik et al., 1993).

Epidemiology studies have identified an increase in allergy, allergic rhinitis, asthma, and asthma-like symptoms (e.g., wheezing and long-term cough) among people and children exposed to airborne fungi in home or school environments (Meng et al., 2012; Portnoy et al., 2005; Seltzer and Fedoruk, 2007; Taskinen et al., 1997; Versalovic et al., 2011). Positive reactions to skin-

prick tests (Gioulekas et al., 2004; Taskinen et al., 1997) and the Phadebas RASTTM technique (Karlsson-Borga et al., 1989) indicate that some of these asthma cases may be caused by *Aspergillus* species. Meta-analysis indicates that approximately 28% of asthmatics have aspergillus hypersensitivity (AH) (Agarwal, 2009). Although *A. tenuis* was isolated in only 4% of the homes in a home study of patients with allergic rhinitis and asthma, 50% of the study population had a positive skin reaction to the molds (Tarlo et al., 1988). Other *Aspergillus* species were also isolated, but more infrequently; *A. fumigatus* was isolated in only 3% of the homes, and *A. flavus* and *A. niger* in <1% of the homes. However, positive skin prick tests for patients in the study were common (36% for *A. fumigatus* and *A. flavus*, and 21% for *A. niger*). Environmental exposure to *Aspergillus* spores is not significantly associated with an increase in the number of hospital admissions among children with asthma (Atkinson et al., 2006). However, indoor levels of culturable *Aspergillus* have a non-linear relationship with asthma symptoms in children based on a study on home samples; the authors hypothesized that the reason for the nonlinearity was the number of different *Aspergillus* species that are commonly found in indoor environments (IOM, 2000; Su et al., 1992).

Numerous cases of asthma have been associated with occupational exposure to *Aspergillus*. Restrictive and obstructive respiratory impairments, specifically post-shift decrements in pulmonary function tests, allergic symptoms, and high IgE levels, were identified in grain storage workers and associated with spores of *Aspergillus*, *Alternaria*, *Drechslera*, *Epicoccum*, *Nigrospora*, and *Periconia* (Chattopadhyay et al., 2007). Baker's Asthma is also associated with an allergen from *A. oryzae*, which is used to produce amylases used by professional bakers (Kilch, 2009), and with exposure to airborne grain flour contaminated with molds, including *Aspergillus* (De Lucca, 2007).

Allergic bronchopulmonary aspergillosis (ABPA) is an allergic pulmonary disease that is characterized by eosinophilia, an increase in the number of eosinophils in the blood, and IgG and IgE antibodies to *Aspergillus*, followed by bronchiectasis, or abnormal widening of the bronchi, and then moderate to severe asthma, bronchial obstruction and other tuberculosis-like symptoms (EPA, 2004; Kilch, 2009; Mazur and Kim, 2006). It generally occurs in cystic fibrosis patients (prevalence of 2-15%) and asthmatics (prevalence of 1-2%) whose airways become colonized and develop subsequent hypersensitivity to *Aspergillus* (Agarwal, 2009; EPA, 2004; Mazur and Kim, 2006). The anatomic changes caused by these diseases are a larger risk factor for development of ABPA than exposure to the mold itself (Brown et al., 2006). This disease is not invasive, but is instead caused by colonization of the respiratory tract (Mazur and Kim, 2006) and exposure to conidia or aspergillus-antigens, usually *A. fumigatus* (Binder and Lass-Flörl, 2013; Kilch, 2009; Mazur and Kim, 2006).

Aspergillus species, specifically *A. flavus*, *A. fumigatus*, *A. niger*, *A. sydowii*, *A. terreus*, and *A. versicolor*, have been isolated repeatedly from patients with allergic fungal sinusitis (AFS), also

known as eosinophilic fungal rhinosinusitis (Binder and Lass-Flörl, 2013; Noble et al., 1997; Seltzer and Fedoruk, 2007). AFS is similar to ABPA; it is a localized hypersensitivity caused by fungal growth in abnormal tissue (Brown et al., 2006; citing Luong et al., 2004) or poor drainage of the sinus cavity (ACOEM, 2002). It is described as a combination of nasal polyposis (development of internal polyps), crust formation, and sinus cultures that have tested positive for fungal infection (Mazur and Kim, 2006). Approximately 13% of cultures taken from AFS patients indicated the presence of *Aspergillus* (Mazur and Kim, 2006).

Hypersensitivity pneumonitis (HP), or allergic alveolitis, is a disease characterized by flu-like symptoms, coughing, and temporary chest tightness; it has been associated with inhalation of *Aspergillus* spores in both adults and children (Binder and Lass-Flörl, 2013; GAO, 2008; Kilch, 2009; Mazur and Kim, 2006; Seltzer and Fedoruk, 2007). HP generally occurs in occupational settings and has been given multiple colloquial names such as “malt worker’s lung”, air-conditioning disease, and farmer’s lung (Kilch, 2009).

Allergic responses to *Aspergillus* exposure may not be limited to the respiratory tract. *Aspergillus* hypersensitivity (AH) causes immediate cutaneous reactions following exposure to *A. fumigatus* antigens (Agarwal, 2009). Only some patients with AH will develop respiratory hypersensitivity (e.g., ABPA) (Agarwal, 2009). A 35 year old man developed contact urticaria, specifically erythema (redness) on the hands and face and wheezing, following contact with mold on the skin of salami casings. Skin prick tests indicated sensitization to *Aspergillus* and *Hormodendrum* (Maibach, 1995).

Overall, *Aspergillus* is a known allergen and is associated with asthma, hypersensitivity, and other inflammatory diseases such as HP, contact urticaria, ABPA, and AFS.

3.3.1.5 Systemic Effects

Aspergillus produces a number of toxic metabolites called mycotoxins, specifically aflatoxin, ochratoxin, sterigmatocysin, cyclopiazonic acid, gliotoxin, patulin, citrinin, and penicillic acid (Kilch, 2009). See sections 4.1, 4.5, 4.6, and 4.7 for more information on aflatoxin, citrinin, ochratoxin, and sterigmatocysin, respectively. Effects of other mycotoxins produced by *Aspergillus* are described here briefly.

Cyclopiazonic acid is produced by *A. flavus*, *A. lentulus*, *A. minisclerotigenes*, *A. oryzae*, *A. pseudotamarii*, and *A. versicolor*. It is associated with disruption of calcium transport, immunity suppression, hepatic cell necrosis, muscular necrosis, and intestinal hemorrhage and edema (Kilch, 2009).

Gliotoxins produced by *A. flavus*, *A. fumigatus*, *A. niger*, *A. terreus*, *Eurotium chevalieri*, *Eu. Rubrum*, and *Neosartorya pseudofischeri* affect immunity and induce cellular apoptosis (Kilch, 2009; Scharf et al., 2012). The presence of gliotoxin is likely a virulence factor of human

mycoses (Kilch, 2009), because it suppresses the immune system by inhibiting neutrophil phagocytosis and apoptosis in macrophages (Kilch, 2009).

Patulin is a toxin produced by *A. clavatus*, *A. langivesica*, and *A. terreus* and commonly found on moldy animal feed and apples. It is associated with serious health effects, including pulmonary and cerebral edema, nausea, gastritis, paralysis, convulsions, capillary damage, and cancer (Kilch, 2009).

Penicillic acid is commonly found in stored grains, cereals, and beans. It is produced by *A. melleus*, *A. ochraceus*, and *A. ostianus* and is associated with a number of severe health effects, including tremors and liver and kidney damage. It also dilates blood vessels and is an antidiuretic (Kilch, 2009).

Although it produces a number of aflatoxins that may be hazardous to human health, no information was located on the potential for *Aspergillus* itself to cause systemic effects.

3.3.2 Health Effects in Animals

Animal models, particularly guinea pigs, rabbits, and mice, have been used to study invasive pulmonary aspergillosis (IPA) and fungal keratitis in both normal and immunosuppressed animals (Clemons and Stevens, 2005). A study in guinea pigs determined that there was no difference in the ability of aspergillopepsin (PEP)- deficient mutants and wild-type *A. fumigatus* to invade tissue and cause mortality (Clemons and Stevens, 2005). In another study, rabbits were given glucocorticoids subconjunctivally to alter the course of *Aspergillus* infection; non-suppressed animals did not develop a detectable fungal burden in their cornea within 7 days; corticoid treated rabbits developed corneal infections throughout the 15 day study and the inflammatory response in their corneas worsened (Clemons and Stevens, 2005).

Murine (mouse) models have also been used to study systemic infection. Immunosuppressed animals inoculated intranasally or intratracheally developed infection; central nervous system (CNS) infection was the most common extra-pulmonary site of infection (resulting in >80% mortality) (Clemons and Stevens, 2005). Murine models for allergic bronchopulmonary aspergillosis (ABPA) indicate that the cause of disease is host-response after sensitization to *Aspergillus* antigens rather than invasive infection (Clemons and Stevens, 2005).

One study reported some systemic toxicity or effects following intraperitoneal (ip) injection of *A. niger*, *A. ruber*, *A. amstelodami*, *A. versicolor*, *A. flavus*, and *A. ochraceus* cultures isolated from cured and noncured tobacco into mice (Hamilton et al., 1969); however, ip injection is not an environmentally relevant route.

Overall, *Aspergillus* exposure in animals is linked to superficial infections at the site of contact (e.g., lungs, skin, or eye) and invasive or systemic infections.

3.3.3 References

ACOEM, 2002. Adverse human health effects associated with molds in the indoor environment. American College of Occupational and Environmental Medicine.

Agarwal, R., 2009. Allergic Bronchopulmonary Aspergillosis. Chest. 135, 805-826.

Ascioglu, S., Rex, J. H., de Pauw, B., Bennett, J. E., Bille, J., Crokaert, F., Denning, D. W., Donnelly, J. P., Edwards, J. E., Erjavec, Z., Fiere, D., Lortholary, O., Maertens, J., Meis, J. F., Patterson, T. F., Ritter, J., Selleslag, D., Shah, P. M., Stevens, D. A., Walsh, T. J., 2002. Defining opportunistic invasive fungal infections in immunocompromised patients with cancer and hematopoietic stem cell transplants: An international consensus. Clin Infect Dis. 34, 7–14.

Atkinson, R. W., Strachan, D. P., Anderson, H. R., Hajat, S., Emberlin, J., 2006. Temporal associations between daily counts of fungal spores and asthma exacerbations. Occup Environ Med. 63, 580-590.

Aznar, C., de Bievre, C., Guiguen, C., 1989. Maxillary sinusitis from *Microascus cinereus* and *Aspergillus repens*. Mycopathologia. 105, 93-97.

Ben-Ami, R., Lewis, R. E., Kontoyiannis, D. P., 2010. Enemy of the (immunosuppressed) state: An update on the pathogenesis of *Aspergillus fumigatus* infection. Br J Haematol. 150, 406-417

Binder, U., Lass-Flörl, C., 2013. New insights into invasive aspergillosis--from the pathogen to the disease. Curr Pharm Des. 19, 3679-3688.

Brakhage, A. A., 2005. Systemic fungal infections caused by *Aspergillus* species: epidemiology, infection process and virulence determinants. Curr Drug Targets. 6, 875-886.

Brakhage, A. A., Liebmann, B., 2005. *Aspergillus fumigatus* conidial pigment and cAMP signal transduction: significance for virulence. Med Mycol. 43, 75-82.

Chattopadhyay, B. P., Das, S., Adhikari, A., Alam, J., 2007. Exposure to varying concentration of fungal spores in grain storage godowns and its effect on the respiratory function status among the workers. Ind Health. 45, 449-461.

Clemons, K. V., Stevens, D. A., 2005. The contribution of animal models of aspergillosis to understanding pathogenesis, therapy and virulence. Med Mycol. 43, 101-110.

De Lucca, A. J., 2007. Harmful fungi in both agriculture and medicine. Rev Iberoam Micol. 24, 3-13.

De Pauw, B., Walsh, T. J., Donnelly, J. P., Stevens, D. A., Edwards, J. E., Calandra, T., Pappas, P. G., Maertens, J., Lortholary, O., Kauffman, C. A., Denning, D. W., Patterson, T. F., Maschmeyer, G., Bille, J., Dismukes, W. E., Herbrecht, R., Hope, W. W., Kibbler, C. C.,

Kullberg, B. J., Marr, K. A., Munoz, P., Odds, F. C., Perfect, J. R., Restrepo, A., Ruhnke, M., Segal, B. H., Sobel, J. D., Sorrell, T. C., Viscoli, C., Wingard, J. R., Zaoutis, T., Bennett, J. E., 2008. Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. Clin Infect Dis. 46, 1813-1821.

EPA (U.S. Environmental Protection Agency), 2004. Guidance for clinicians on the recognition and management of health effects related to mold exposure and moisture indoors. United States Environmental Protection Agency.

GAO (Government Accountability Office), 2008. Indoor Mold: Better coordination of research on health effects and more consistent guidance would improve federal efforts. United States Government Accountability Office.

Gioulekas, D., Damialis, A., Papakosta, D., Spijsma, F., Giouleka, P., Patakas, D., 2004. Allergenic fungi spore records (15 years) and sensitization in patients with respiratory allergy in Thessaloniki-Greece. J Investig Allergol Clin Immunol. 14, 225-231.

Hamilton, P. B., Lucas, G. B., Welty, R. E., 1969. Mouse toxicity of fungi of tobacco. Appl Microbiol. 18, 570-574.

Hedayati, M. T., Pasqualotto, A. C., Warn, P. A., Bowyer, P., Denning, D. W., 2007. *Aspergillus flavus*: Human pathogen, allergen and mycotoxin producer. Microbiology. 153, 1677-1692.

Hope, W. W., Walsh, T. J., Denning, D. W., 2005. Laboratory diagnosis of invasive aspergillosis. Lancet Infect Dis. 5, 609-622.

HUD, 2006. Healthy Homes Issues: Mold. U.S. Department of Housing and Urban Development.

IOM, 2000. Clearing the air: asthma and indoor air exposures. Institute of Medicine, National Academy Press, Washington, D.C.

Karlsson-Borga, A., Jonsson, P., Rolfsen, W., 1989. Specific IgE antibodies to 16 widespread mold genera in patients with suspected mold allergy. Ann Allergy. 63, 521-526.

Klich, M. A., 2009. Health effects of *Aspergillus* in food and air. Toxicol Ind Health. 25, 657-667.

Lass-Flörl, C., Rath, P., Niederwieser, D., Kofler, G., Würzner, R., Krezy, A., Dierich, M. P., 2000 *Aspergillus terreus* infections in haematological malignancies: Molecular epidemiology suggests association with in-hospital plants. J Hosp Infect. 46, 31-35.

- Maibach, H. I., 1995. Contact urticaria syndrom from mold on salami casing. contact dermatitis. 32, 121.
- Marques, S. A., Robles, A. M., Tortorano, A. M., Tuculet, M. A., Negroni, R., Mendes, R. P., 2000. Mycoses associated with AIDS in the Third World. Med Mycol. 38, 269-279.
- Mazur, L. J., Kim, J., Committee on Environmental Health, American Academy of Pediatrics. 2006. Spectrum of noninfectious health effects from molds. Pediatrics. 118, e1909-1926.
- Meng, J., Barnes, C. S., Rosenwasser, L. J., 2012. Identity of the fungal species present in the homes of asthmatic children. Clin Exp Allergy. 42, 1448-1458.
- Mylonakis, E., Paliou, M., Sax, P. E., Skolnik, P. R., Baron, M. J., Rich, J. D., 2000. Central nervous system aspergillosis in patients with human immunodeficiency virus infection. Report of 6 cases and review. Medicine (Baltimore). 79, 269-280.
- Noble, J. A., Crow, S. A., Ahearn, D. G., Kuhn, F. A., 1997. Allergic fungal sinusitis in the southeastern USA: involvement of a new agent *Epicoccum nigrum* Ehrenb. ex Schlecht. 1824. J Med Vet Mycol. 35, 405-409.
- Portnoy, J. M., Kwak, K., Dowling, P., VanOsdol, T., Barnes, C., 2005. Health effects of indoor fungi. Ann Allergy Asthma Immunol. 94, 313-319.
- Rizzetto, L., Cavalieri, D., 2011. Friend or foe: using systems biology to elucidate interactions between fungi and their hosts. Trends Microbiol. 19, 509-515.
- Scharf, D. H., Heinekamp, T., Remme, N., Hortschansky, P., Brakhage, A. A., Hertweck, C., 2012. Biosynthesis and function of gliotoxin in *Aspergillus fumigatus*. Appl Microbiol Biotechnol. 93, 467-472.
- Seltzer, J. M., Fedoruk, M. J., 2007. Health effects of mold in children. Pediatr Clin N Am. 54, 309-333.
- Shinn, E. A., Griffin, D. W., 2003. Atmospheric transport of mold spores in clouds of desert dust. Archives of Environmental Health. 498-504.
- Sporik, R. B., Arruda, L. K., Woodfolk, J., Chapman, M. D., Platts-Mills, T. A., 1993. Environmental exposure to *Aspergillus fumigatus* allergen (Asp f I). Clin Exp Allergy. 23, 326-331.
- Su, H. J., Rotnitzky, A., Burge, H. A., Spengler, J. D., 1992. Examination of fungi in domestic interiors by using factor analysis: Correlations and associations with home factors. Appl Environ Microbiol. 58, 181-186.
- Tarlo, S. M., Fradkin, A., Tobin, R. S., 1988. Skin testing with extracts of fungal species derived

from the homes of allergy clinic patients in Toronto, Canada. Clin Allergy. 18, 45-52.

Taskinen, T., Meklin, T., Nousiainen, M., Husman, T., Nevalainen, A., Korppi, M., 1997. Moisture and mould problems in schools and respiratory manifestations in schoolchildren: clinical and skin test findings. Acta Paediatr. 86, 1181-1187.

Versalovic, J., Carroll, K. C., Funke, G., Jorgensen, J. H., Landry, M. L., Warnock, D. W. Eds.), 2011. Manual of Clinical Microbiology. 10th Edition, Vol. 2. . ASM Press, Washington, D.C.

3.4 Chaetomium

3.4.1 Uptake and Systemic Spread

Members of the genus *Chaetomium* are reported to be the third most common indoor fungal contaminant and are also found in soil, air and plant debris (Pieckova, 2003; Zhang et al., 2012). Exposure to *Chaetomium* is through breaks or wounds in the skin and after corneal trauma. Inhalation is another route of exposure because *Chaetomium* species produce spores. Infections beyond the site of contact (systemic effects) with *Chaetomium* are found in people with compromised health, such as transplant patients, drug users, or otherwise immunocompromised patients (Barron et al., 2003).

3.4.2 Health Effects in Humans

3.4.2.1 Site of Contact Effects

Chaetomium contain melanin pigments in their cell wall and are associated with a heterogeneous group of mycotic infections (Hubka et al., 2011). The most commonly reported infections are subcutaneous or superficial cutaneous and onychomycoses (fungal infection of the nails). However, there are reports of mycotic (fungal) otitis externa and keratitis (infection of the cornea). *Chaetomium* infections are usually not the primary insult; they are a secondary result associated with another problem, such as disease observed in posttraumatic immunocompetent patients (Hubka et al., 2011).

Hubka et al. (2011) provides a review of dermatomycotic *Chaetomium* infections (fungal infection of the skin). The majority of the skin infections are associated with *C. globosum*, *C. funicola*, and *C. murorum* (Tullio et al., 2010; Costa et al., 1988; Piepenbring et al., 2007). In addition, *C. atrobrunneum* was reported to cause mycotic keratitis (Balne et al., 2012). Onychomycosis caused by an unidentified *Chaetomium* species was reported in a case study report from an 11-year old girl (Falcon et al., 2008) and from a 48-year-old male (Hubka et al., 2011).

Chaetomium infection in the sinuses was found in a patient who was being treated for pain and excessive secretion from her sinuses (Aru et al., 1997). *C. brasiliense* was found in a patient with otitis externa (Hubka et al., 2011).

3.4.2.2 Irritation

No information was located that identified *Chaetomium* species as a cause of irritation.

3.4.2.3 Sensitization, Inflammation, Allergy and Asthma

A slightly higher level of sensitization to *C. globosum* was found in patients with moderate or severe asthma, compared to those with mild asthma as tested by skin pricks (Niedoszytko, 2007). It was not clear whether the difference was statistically significant. An association between *Chaetomium* and sensitization, inflammation, allergy or asthma was found in a study where dust from vacuum bags was analyzed for mold DNA. *C. globosum* was identified as one of the primary species found in the homes of asthmatics as reported in this study (Vesper et al., 2007). Overall, in the literature there is no substantial evidence to support a role for *Chaetomium* in sensitization, inflammation, allergy or asthma.

3.4.2.4 Systemic Effects

Chaetomium species are opportunistic pathogens, and patients with impaired immunity are susceptible to infection by *Chaetomium*. Infection is believed to be a result of direct inoculation and then spread hematogenously or disseminated through the blood stream.

Invasive *Chaetomium* infections have been reported in the brain and the lung (Barron et al., 2003). An additional case study reported a patient who had an infection of the myocardium in addition to the brain and lung (Hubka et al., 2011). In all these cases the exposure route was not reported.

In a case study report *C. perlucidum* infected the lungs, brain and myocardium in a patient with acute myelogenous leukemia (Barron et al., 2003). Upon autopsy the invasive fungal infection was determined and later identified. *C. perlucidum* was also found in the lung from a patient with asthma and chronic bronchiectasis. Other *Chaetomium* species identified in the lung are *C. globosum* and *C. atrobrunneum*. They were identified from immunocompromised patients with leukemia, lymphoma or myeloma (Barron et al., 2003).

Pieckova (2003) reported *Chaetomium* infection in four patients after bone marrow transplantation due to the presence of *Chaetomium* in the hospital. *Chaetomium* species known to cause systemic infections include *C. atrobrunneum*, *C. perlucidum*, and *C. strumarium*. *C. globosum* and *C. atrobrunneum* infections have been implicated in causing cerebral mycosis in identified drug users (Abbott et al., 1995) and a renal transplant patient (Anandi et al., 1989). *C. atrobrunneum* was also found in the brain and lung of a patient with multiple myeloma (plasma cell tumor) undergoing a bone marrow transplant and in another patient undergoing renal transplant (Barron et al., 2003).

3.4.3 Health Effects in Animals

Only one report of *Chaetomium* associated with skin infection in a dog has been reported (Hubka et al., 2011).

3.4.4 References

Abbott, S. P., Sigler, L., McAleer, R., McGough, D. A., Rinaldi, M. G., Mizell, G., 1995. Fatal cerebral mycoses caused by the ascomycete *Chaetomium strumarium*. J Clin Microbiol. 33, 2692-2698. Abstract only.

Anandi, V., John, T. J., Walter, A., Shastry, J. C., Lalitha, M. K., Padhye, A. A., Ajello, L., Chandler, F. W., 1989. Cerebral phaeohyphomycosis caused by *Chaetomium globosum* in a renal transplant recipient. J Clin Microbiol. 27, 2226-2229. Abstract only.

Aru, A., Munk-Nielsen, L., Federspiel, B. H., 1997. The soil fungus *Chaetomium* in the human paranasal sinuses. Eur Arch Otorhinolaryngol. 254, 350-352. Abstract only.

Balne, P. K., Nalamada, S., Kodiganti, M., Taneja, M., 2012. Fungal keratitis caused by *Chaetomium atrobrunneum*. Cornea. 31, 94-95.

Barron, M. A., Sutton, D. A., Veve, R., Guarro, J., Rinaldi, M., Thompson, E., Cagnoni, P. J., Moultny, K., Madinger, N. E., 2003. Invasive mycotic infections caused by *Chaetomium perlucidum*, a new agent of cerebral phaeohyphomycosis. J Clin Microbiol. 41, 5302-5307.

Costa, A. R., Porto, E., Lacaz Cda, S., de Melo, N. T., Calux Mde, J., Valente, N. Y., 1988. Cutaneous and ungual phaeohyphomycosis caused by species of *Chaetomium* Kunze (1817) ex Fresenius, 1829. J Med Vet Mycol. 26, 261-268. Abstract only.

Falcon, C. S., Falcon Mdel, M., Ceballos, J. D., Florencio, V. D., Erchiga, V. C., xOrtega, S. C., 2008. Onychomycosis by *Chaetomium* spp. Mycoses. 52, 77-79.

Hubka, V., Mencl, K., Skorepova, M., Lyskova, P., Zalabska, E., 2011. Phaeohyphomycosis and onychomycosis due to *Chaetomium* spp., including the first report of *Chaetomium brasiliense* infection. Med Mycol. 49, 724-733.

Niedoszytko, M., Chelmińska, M., Jassem, E., Czestochowska, E., 2007. Association between sensitization to *Aureobasidium pullulans* (*Pullularia* sp) and severity of asthma. Ann Allergy Asthma Immunol. 98, 153-156.

Pieckova, E., 2003. In vitro toxicity of indoor *Chaetomium* Kunze ex Fr. Ann Agric Environ Med. 10, 9-14.

Piepenbring, M., Caceres Mendez, O. A., Espino Espinoza, A. A., Kirschner, R., Schofer, H., 2007. Chromoblastomycosis caused by *Chaetomium funicola*: a case report from Western

Panama. Br J Dermatol. 157, 1025-1029. Abstract only.

Tullio, V., Banche, G., Allizond, V., Roana, J., Mandras, N., Scalas, D., Panzone, M., Cervetti, O., Valle, S., Carlone, N., Cuffini, A. M., 2010. Non-dermatophyte moulds as skin and nail foot mycosis agents: *Phoma herbarum*, *Chaetomium globosum* and *Microascus cinereus*. Fungal Biol. 114, 345-349.

Vesper, S., McKinstry, C., Ashley, P., Haugland, R., Yeatts, K., Bradham, K., Svendsen, E., 2007. Quantitative PCR analysis of molds in the dust from homes of asthmatic children in North Carolina. J Environ Monit. 9, 826-830. Abstract only.

Zhang, Q., Li, H. Q., Zong, S. C., Gao, J. M., Zhang, A. L., 2012. Chemical and bioactive diversities of the genus *Chaetomium* secondary metabolites. Mini Rev Med Chem. 12, 127-148.

3.5 Cladosporium

3.5.1 Uptake and Systemic Spread

Members of the genus *Cladosporium* have worldwide distribution and are one of the most common indoor fungal contaminants found in the air and on surfaces, particularly in moist conditions. *Cladosporium* are also found in soil, air and plant debris outdoors (CDC, 2012; Qiu-Xia et al., 2007). *Cladosporium* is an opportunistic pathogen, and patients with impaired immunity are susceptible to infection by *Cladosporium*. Exposure to *Cladosporium* can occur via breaks or wounds in the skin or corneal abrasions. In addition, *Cladosporium* species produce spores and, thus, exposure also occurs by inhalation. *Cladosporium* can cause infections affecting the skin, eye and pulmonary system (CDC, 2012). *Cladosporium* systemic infections are rare, with little evidence that supports anticipated systemic spread. Of the systemic effect cases reported, the portal of entry is often unknown.

3.5.2 Health Effects in Humans

3.5.2.1 Site of Contact Effects

Subcutaneous or cutaneous infections by *C. sphaerospermum* (Qiu-Xia et al., 2007), *C. oxysporum* (Romano et al., 1999), *C. bantiana* (Patterson et al., 1999) and *C. cladosporioides* (Sang et al., 2011) have been reported. *C. carrionii* has been associated with the infection of fingernails (Barde and Singh, 1984). *C. cladosporioides* has also been associated with an infection of the eye that occurred after a corneal laceration (Chew et al., 2009). This species was also found growing in the upper right lobe of the lung without invasion into the adjacent tissue in a patient with severe diabetes mellitus (Kwon-Chung et al., 1975). *Alternaria* and *Cladosporium* were identified from fruit handled at an Italian worker's workplace. This patient had developed hypo- and hyper-pigmented areas on his hands after handling mold-covered fruit (Guarneri et al., 2008). The pigment alterations were attributed to intracutaneous penetration of the melanoid fungal pigment, but this suspicion was not further evaluated.

3.5.2.2 Irritation

No information was located that identified *Cladosporium* species as a cause of irritation.

3.5.2.3 Sensitization, Inflammation, Allergy and Asthma

The inhalation of fungi and their spores is a well-established cause of upper and lower respiratory allergic reaction in sensitized subjects (D'Amato et al., 1997). In a European clinical trial, approximately 900 patients with allergic respiratory symptoms were tested for sensitivity by the skin prick test to determine serum IgE to *Alternaria* and *Cladosporium*. Approximately 10% of the subjects tested positive for sensitivity to *Cladosporium* (D'Amato et al., 1997). Another study of approximately 10,000 participants was conducted to identify factors associated with severe asthma (Cazzoletti et al., 2010). Sensitization to *Cladosporium* was an important determinant of asthma severity (Cazzoletti et al., 2009). The study concluded that sensitization to *Cladosporium* was associated with a more than 5-fold greater risk of having mild, moderate or severe persistent asthma than intermittent asthma. In a previous study on the Isle of Wight (United Kingdom), *Cladosporium* sensitization was found to develop in children by the age of 4 and was correlated with diagnoses of asthma, eczema and rhinitis (Tariq et al., 1996).

Cladosporium species can trigger allergic reactions in sensitive individuals. Concentrations of 400 to 500 CFU/m³ were reported both indoors and outdoors in a study of a low-income area of Syracuse, NY; concentrations in the air were the highest in the summer and fall (Crawford et al., 2015). Prolonged exposure to elevated spore concentrations can elicit chronic allergy and asthma. *Cladosporium herbarum* is considered the most important allergenic species, and epidemiology studies have associated it with the development, persistence and severity of asthma (Knutsen et al., 2012). Sensitivity to fungal allergens, including *Cladosporium*, has been associated with cases of life threatening asthma attacks (Black et al., 2000). The patient described above in the context of the cutaneous case also experienced asthma attacks, but after the cutaneous infection was cured, no further episodes of asthma were reported, supporting a link between the fungal infection and asthma (Guarneri et al., 2008).

In a study evaluating 34 patients with various interstitial lung diseases, 17 patients with bronchial asthma and 21 control subjects, a significantly higher tendency for high anti-*Cladosporium* antibody titers were found in the interstitial lung diseases group (12 patients out of 34 patients) compared to patients with bronchial asthma (0/17) or control subjects (0/21), suggesting *Cladosporium* could be involved in the development of interstitial lung diseases (Watanuki et al., 2012). The results of these investigations clearly document and support the conclusion that *Cladosporium* exposure and subsequent sensitization play a role in allergic respiratory disease including asthma. This conclusion is also supported by a number of studies with experimental animal models

There is evidence supporting the conclusion that *Cladosporium* is associated with allergic reactions, interstitial lung diseases and asthma.

3.5.2.4 Systemic Effects

Palaoglu et al. (1993) reported a fatal fungal infection of the brain in a 14 year old Turkish female with no evidence of a portal of entry. A brain infection with *Cladosporium bantiana* (also called *Cladophialophora bantiana*) has also been reported in a 38 year old male located in China (Huang et al., 2011). The latter patient was a farmer with a history of non-insulin diabetes and myelodysplastic syndrome (ineffective production of blood cells) and was admitted to the hospital following faciobrachial tonic seizure and right-sided hemiparesis. He was unresponsive to treatment and died after discharge from the hospital. It is speculated that the portal of entry was inhalation of *Cladosporium* in soil. Shields and Castillo (2002) reported on a case of *C. bantiana* infection of the spinal cord in a patient from North Carolina on steroids for the treatment of sarcoidosis (abnormal collections of inflammatory cells [granulomas] that form nodules in organs, particularly lungs). The patient presented with back pain, and the fungal infection in the spinal cord caused transverse myelitis or spinal cord dysfunction, resulting in the inability to walk. There was no evidence of a portal of entry. The patient was successfully treated and recovered.

A rare case of pulmonary infection by *Cladosporium cladosporioides* was reported in an immune-competent patient (Castro et al., 2013). In this case, a 27 year-old female was believed to be exposed at work via inhalation while performing quality control on cork. Her symptoms were initially mild with a dry cough, malaise and low fever that progressed to persistent fever and malaise. Following treatment for fungal infection with voriconazole, she made a complete recovery. A bronchial infection with *Cladosporium sphaerospermum* in a healthy non-asthmatic 58 year-old woman was reported in 2003 (Yano et al., 2003). A persistent dry cough was the only symptom but a chest radiograph identified nodular opacity in her bronchus. Following site-specific treatment, the patient improved, as demonstrated by subsequent chest radiographs. These two cases demonstrate that unusual cases of systemic infection can occur in healthy immune-competent patients.

The systemic effects identified in the literature have been reported in the brain, lung and the spinal cord. *Cladosporium bantiana* is recognized as a cause of CNS infection in immunosuppressed patients (Shields and Castillo, 2002).

3.5.3 Health Effects in Animals

No standard studies were identified that investigated the effects of *Cladosporium* in standard experimental models. *Cladosporium* is commonly found on the skin and hair of cats and dogs (Jang et al., 2007; Bernardo et al., 2005) and appears to cause opportunistic infections. For example, cerebral phaeohyphomycosis (opportunistic infection caused by dematiaceous or dark walled fungi) has been reported in dogs and cats (Dillehay et al., 1987). Clinically affected dogs usually present with systemic illness, characterized by vague symptoms, such as fever and

malaise. Behavioral changes were observed in a dog with *Cladosporium trichoides* infection in the cerebellum, liver, kidney and spleen (Newsholme et al., 1980). A German shepherd dog was reported to have granulomatous encephalitis and nephritis due to a *Cladosporium cladosporioides* infection (Poutahidis et al., 2009).

Cladosporium herbarum is a potent inducer of IgE production in mice, consistent with the allergic effects observed in humans. Mice sensitized by prior intraperitoneal (ip) exposure to *C. herbarum* spores developed allergic lung inflammation and hyper-reactivity. Furthermore, intranasal administration of *C. herbarum* spores for 7 weeks to unsensitized BALB/c mice increased total serum IgE and the appearance of specific IgE. The intranasal exposure also resulted in sensitization of the previously unsensitized mice, as evidenced by the appearance of airway hyper-reactivity in response to methacholine challenge. The hyperreactivity appeared within 3 weeks and continued for the entire 10-12 week period of treatment with the *C. herbarum* spores (Denis et al., 2007). Topical application or subcutaneous administration of *C. sphaerospermum* to BALBc and immunocompromised BALBc mice caused a higher number and larger skin lesions in the immunocompromised mice. The immunocompromised mice died within 5 days of intravenous administration, whereas the competent mice survived for 5 weeks (Huyan et al., 2012). Other studies in mice and guinea pigs showed that the spores of *C. cladosporioides* are more allergenic than the mycelium (Bouziane et al., 2005). These experimental results in mice directly confirm the finding of allergic reactions to *Cladosporium* in humans, as well as sensitization as a result of prior exposure and the increased risk of immunocompromised individuals.

3.5.4 References

- Barde, A. K., Singh, S. M., 1984. *Cladosporium carrionii* Trejos 1954 infection of human nail. Mykosen. 27, 366-369.
- Bernardo, F., Lança, A., Guerra, M. M., Marina Martins, H., 2005. Dermatophytes isolated from pet, dogs and cats, in Lisbon, Portugal (2000-2004). RPCV (Revista Portuguesa de Ciencias Veterinarias). 100, 85-88.
- Black, P. N., Udy, A. A., Brodie, S. M., 2000 Sensitivity to fungal allergens is a risk factor for life-threatening asthma. Allergy. 55, 501-504.
- Bouziane, H., Latgé, J. P., Fitting, C., Mecheri, S., Lelong, M., David, B., 2005 Comparison of the allergenic potency of spores and mycelium of *Cladosporium*. Allergol Immunopathol (Madr). 33, 125-130.
- Castro, A. S., Oliveira, A., Lopes, V. 2013 Pulmonary phaeohyphomycosis: A challenge to the clinician. Eur Respir Rev. 22, 187–192. Letter to the editor.

Cazzoletti, L., Marcon, A., Corsico, A., Janson, C., Jarvis, D., Pin, I., Accordini, S., Bugiani, M., Cerveri, I., Gislason, D., Gulsvik, A., de Marco, R., Therapy and Health Economics Group of the European Community Respiratory Health Survey, 2010. Asthma severity according to Global Initiative for Asthma and its determinants: an international study. *Int Arch Allergy Immunol.* 151, 70-79.

CDC (Centers for Disease Control and Prevention), 2012. *Cladosporium*: Other Pathogenic Fungi. Centers for Disease Control and Prevention. Available at ; <http://www.cdc.gov/fungal/diseases/other/cladosporium.html>

Chew, F. L., Subrayan, V., Chong, P. P., Goh, M. C., Ng, K. P., 2009. *Cladosporium cladosporioides* Keratomycosis: A case report. *Jpn J Ophthalmol.* 53, 657-659.

Crawford, J. A., Rosenbaum, P. F., Anagnost, S. E., Hunt, A., Abraham, J. L., 2015. Indicators of airborne fungal concentrations in urban homes: Understanding the conditions that affect indoor fungal exposures. *Sci Total Environ.* 517, 113-124.

D'Amato, G., Chatzigeorgiou, G., Corsico, R., Gioulekas, D., Jäger, L., Jäger, S., Kontou-Fili, K., Kouridakis, S., Liccardi, G., Meriggi, A., Palma-Carlos, A., Palma-Carlos, M. L., Pagan Aleman, A., Parmiani, S., Puccinelli, P., Russo, M., Spieksma, F., Torricelli, R., Wüthrich, B., 1997. Evaluation of the prevalence of skin prick test positivity to *Alternaria* and *Cladosporium* in patients with suspected respiratory allergy. A European multicenter study promoted by the Subcommittee on Aerobiology and Environmental Aspects of Inhalant Allergens of the European Academy of Allergology and Clinical Immunology. *Allergy.* 52, 711-716.

Denis, O., van den Brûle, S., Heymans, J., Havaux, X., Rochard, C., Huaux, F., Huygen, K., 2007. Chronic intranasal administration of mould spores or extracts to unsensitized mice leads to lung allergic inflammation, hyper-reactivity and remodelling. *Immunology.* 122, 268-278.

Dillehay, D. L., Ribas, J. L., Newton, J. C., Kwapien, R. P., 1987. Cerebral phaeohyphomycosis in two dogs and a cat. *Vet Pathol.* 24, 192-194.

Guarneri, F., Guarneri, C., Cannavò, S. P., Guarneri, B., 2008. Dyschromia of hands and bronchial asthma caused by sooty molds. *Am J Clin Dermatol.* 9, 341-343.

Huang, W. M., Fan, Y. M., Li, W., Yang, W. W., 2011. Brain abscess caused by *Cladophialophora bantiana* in China. *J Med Microbiol.* 60, 1872-1874.

Huyan, X. H., Yang, Y. P., Fan, Y. M., Huang, W. M., Li, W., Zhou, Y., 2012. Cutaneous and systemic pathogenicity of a clinical isolate of *Cladosporium sphaerospermum* in a murine model. *J Comp Pathol.* 147, 354-359.

Jang, K.-S., Yun, Y.-H., Yoo, H.-D., Kim S H, 2007. Molds isolated from pet dogs. *Mycobiology.* 35, 100-102.

- Knutsen, A. P., Bush, R. K., Demain, J. G., Denning, D. W., Dixit, A., Fairs, A., Greenberger, P. A., Kariuki, B., Kita, H., Kurup, V. P., Moss, R. B., Niven, R. M., Pashley, C. H., Slavin, R. G., Vijay, H. M., Wardlaw, A. J., 2012. Fungi and allergic lower respiratory tract diseases. *J Allergy Clin Immunol.* 129, 280-291.
- Kwon-Chung, K. J., Schwartz, I. S., Rybak, B. J., 1975. A pulmonary fungus ball produced by *Cladosporium cladosporioides*. *Am J Clin Pathol.* 64, 564-568.
- Newsholme, S. J., Tyrer, M. J., 1980. Cerebral mycosis in a dog caused by *Cladosporium trichoides* Emmons 1952. *Onderstepoort J Vet Res.* 47, 47-49. Abstract only.
- Palaoglu, S., Sav, A., Basak, T., Yalcinlar, Y., Scheithauer, B. W., 1993. Cerebral phaeohyphomycosis. *Neurosurgery.* 33, 894-897.
- Patterson, J. W., Warren, N. G., Kelly, L. W., 1999. Cutaneous phaeohyphomycosis due to *Cladophialophora bantiana*. *J Am Acad Dermatol.* 40, 364-366.
- Poutahidis, T., Angelopoulou, K., Karamanavi, E., Polizopoulou, Z. S., Doulberis, M., Latsari, M., Kaldrymidou, E., 2009. Mycotic encephalitis and nephritis in a dog due to infection with *Cladosporium cladosporioides*. *J Comp Pathol.* 140, 59-63. Abstract only.
- Qiu-Xia, C., Chang-Xing, L., Wen-Ming, H., Jiang-Qiang, S., Wen, L., Shun-Fang, L., 2008. Subcutaneous phaeohyphomycosis caused by *Cladosporium sphaerospermum*. *Mycoses.* 51, 79-80.
- Romano, C., Bilenchi, R., Alessandrini, C., Miracco, C., 1999. Case report. Cutaneous phaeohyphomycosis caused by *Cladosporium oxysporum*. *Mycoses.* 42, 111-115.
- Sang, H., Zheng, X. E., Zhou, W. Q., He, W., Lv, G. X., Shen, Y. N., Kong, Q. T., Liu, W. D., 2012. A case of subcutaneous phaeohyphomycosis caused by *Cladosporium cladosporioides* and its treatment. *Mycoses.* 55, 195-197.
- Shields, G. S., Castillo, M., 2002. Myelitis caused by *Cladophialophora bantiana*. *Am J Roentgenol.* 179, 278-279.
- Tariq, S. M., Matthews, S. M., Stevens, M., Hakim, E. A., 1996. Sensitization to *Alternaria* and *Cladosporium* by the age of 4 years. *Clin Exp Allergy.* 26, 794-798.
- Watanuki, Z., Okada, S., Chiba, S., Kamei, K., Suzuki, Y., Yamada, N., 2012. Increased prevalence of high anti-*Cladosporium* antibody titers in interstitial lung diseases. *Tohoku J Exp Med.* 226, 287-291.
- Yano, S., Koyabashi, K., Kato, K., 2003. Intrabronchial lesion due to *Cladosporium sphaerospermum* in a healthy, non-asthmatic woman. *Mycoses.* 46, 348-350.

3.6 Dicyma

3.6.1 Uptake and Systemic Spread

No information was located on the uptake and systemic spread of *Dicyma*. However, the absence of reports of systemic spread suggests that any health risk from *Dicyma* exposure is primarily from the site of contact.

3.6.2 Health Effects in Humans

Very little information exists on the effects of *Dicyma* species (also called *Ascotricha chartarum* Berk) (American Testing, 2015). However, the references found indicate that *Dicyma* species appear to be a potential source for new drugs. For example, Ascotricins A and B were isolated from *Ascotricha chartarum* Berk. SANK 14186, and are novel sphingosine-1-phosphate receptor 1 (S1P1) antagonists (Yonesu et al., 2009). In addition, a 5-HT inhibitor, an ergoline derivative, was isolated from *Dicyma*.

3.6.2.1 Site of Contact Effects

A 35 year old female farmer in India was confirmed to have a maxillary sinus infection that was identified as *Dicyma ampullifera* Boul (also known as *Ascotricha chartarum* Berk). This was reported as the first case of a sinus infection caused by this fungus (Singh et al., 1996), and occurred following a face injury. The patient's symptoms included a hard bony swelling with mild tenderness. A diagnosis of indolent (chronic and unilateral) sinusitis and allergic sinusitis (no evidence of tissue invasion) was provided for this patient with the caveat that slow progressive invasion to the surrounding area was observed. The patient recovered after treatment with antifungals and surgery.

3.6.2.2 Irritation

No information was located that identified *Dicyma* species as a cause of irritation.

3.6.2.3 Sensitization, Inflammation, Allergy and Asthma

No information was found on the sensitization and allergic potential of *Dicyma*.

3.6.2.4 Systemic Effects

No information was found on systemic effects associated with *Dicyma*.

3.6.3 Health Effects in Animals

No information was found on the association of *Dicyma* with effects in animals.

3.6.4 References

American Air Testing, 2015. Dicyma. Page Dactylaria-Drechslera. Available at http://test.verecom.com/wp_aat/services/toxic-mold/mold-descriptions/dactylaria-drechslera

Singh, S. M., Naidu, J., Jain, S., Nawange, S. R., Dhindsa, M. K., 1996. Maxillary sinusitis caused by *Ascotricha chartarum* Berk. (anamorph *Dicyma ampullifera* Boul.): a new phaeoid opportunistic human pathogen. *J Med Vet Mycol.* 34, 215-218.

Yonesu, K., Ohnuki, T., Ono, Y., Takatsu, T., Nara, F., 2009. Ascotricins A and B, novel antagonists of sphingosine-1-phosphate receptor 1 from *Ascotricha chartarum* Berk. *SANK* 14186. *J Antibiot* (Tokyo). 62, 359-364.

3.7 *Epicoccum*

3.7.1 Uptake and Systemic Spread

No information was located on the potential for *Epicoccum* species to cause systemic effects or produce a toxin. Most reported effects occur at the site of contact (e.g., skin or lung).

3.7.2 Health Effects in Humans

3.7.2.1 *Site of Contact Effects and Local Infection*

Prior to 1987, *Epicoccum* species were not considered to be highly infectious and were less of a concern for infection compared to other pathogenic molds (Pritchard and Muir, 1987). However, in 1997, references began to appear linking *Epicoccum* with skin disease and other health effects (Weber, 2006). For example, phaeohyphomycosis, a loosely defined term that includes skin disease caused by dematiaceous (darkly pigmented) molds, has been associated with *Epicoccum* (Weber 2006).

3.7.2.2 *Irritation*

No information was located on the potential for *Epicoccum* to cause irritation.

3.7.2.3 *Sensitization, Inflammation, Allergy and Asthma*

Epicoccum species are associated with skin reactivity and sensitivity among asthmatic patients (Black et al., 2000; Karlsson-Borga et al., 1989; Niedozytko et al., 2007; Portnoy et al., 1987), although these reactions are smaller in intensity and quantity when compared to other molds (e.g., *Cladosporium*, *Alternaria*, and *Fusarium*) (Tarlo et al. 1988). The positive reactions to skin-prick tests and the Phadebas RAST™ technique indicate that some asthma cases may be caused by *Epicoccum*. Although there is generally a high potential for cross-reactivity among mold species and for multiple mold sensitivity, some research suggests that *Epicoccum* does not share antigens with other genera, and that cross-reactivity with other molds is unlikely (Koivikko

et al. 1991). More recent evidence, however, indicates that there is significant cross-reactivity between *Epicoccum* species and other molds, including *Alternaria alternata*, *Curvularia lunata*, *Cladosporium herbarum*, *Penicillium citrinum*, *Fusarium solani* and *Aspergillus fumigatus* (Bisht et al., 2004; Bisht et al., 2002).

Epidemiology studies have linked *Epicoccum* exposure to asthma and asthma-like symptoms in both children and adults. Environmental exposure to *Epicoccum* spore concentrations of 60 spores/m³ [a 12-hour time-weighted-average (TWA)] were significantly associated with an increased incidence in morning cough and a decrease in morning peak expiratory flow rates (PEFR), the speed at which a person can exhale, among asthmatic children (Neas et al. 1996). However, causality cannot be determined because other mold spores (e.g., *Cladosporium*, and some basidiospores) are also associated with these effects. Environmental *Epicoccum* exposures have also been marginally associated with an increase in the number of hospital admissions among children with asthma; however, there is no dose-response relationship (Atkinson et al., 2006). Asthmatic responses are not associated solely with *Epicoccum* spores in the environment, but also with other spores found in the home. *Cladosporium*, *Penicillium*, and *Aspergillus* were also found in homes with asthmatic children more often than homes without an asthmatic child (Meng et al., 2012).

Epicoccum also commonly infects plants, specifically barley, oats, wheat, and corn (Weber, 2006). Restrictive and obstructive respiratory impairments, specifically post-shift decrements on pulmonary function tests, allergic symptoms, and high IgE levels, were identified in grain storage workers and associated with the presence of *Aspergillus*, *Alternaria*, *Drechslera*, *Epicoccum*, *Nigrospora*, and *Periconia* spores (Chattopadhyay et al., 2007).

Epicoccum is also associated with the development of allergic respiratory disease, specifically hypersensitivity pneumonitis (HP), allergy-related inflammation, and Allergic Fungal Sinusitis (AFS), an allergic reaction to aerosolized fungi. Shower curtains contaminated with *Epicoccum nigrum* are known to cause “shower-curtain lung”, or HP (Mazur and Kim, 2006). *Epicoccum* has also been shown to colonize nasal sinuses and cause AFS (Noble et al., 1997).

Overall, *Epicoccum* species may contribute to new-onset or exacerbation of asthma, but the causal link is neither clear nor quantifiable. It is also associated with other allergic respiratory diseases, including AFS and HP.

3.7.2.4 Systemic Effects

No information was located on the potential for *Epicoccum* to cause systemic effects in humans.

3.7.3 Health Effects in Animals

No reliable information was located on the potential for *Epicoccum* to cause effects in animals. One study (Hamilton et al., 1969) reported lethality among mice exposed to an ip injection of fungal cultures isolated from cured and noncured tobacco into mice (Hamilton et al., 1969); however, ip injection is not an environmentally relevant route.

3.7.4 References

- Atkinson, R. W., Strachan, D. P., Anderson, H. R., Hajat, S., Emberlin, J., 2006. Temporal associations between daily counts of fungal spores and asthma exacerbations. *Occup Environ Med.* 63, 580-90.
- Bisht, V., Arora, N., Singh, B. P., Gaur, S. N., Sridhara, S., 2004. Purification and characterization of a major cross-reactive allergen from *Epicoccum purpurascens*. *Int Arch Allergy Immunol.* 133, 217-24.
- Bisht, V., Singh, B. P., Arora, N., Gaur, S. N., Sridhara, S., 2002. Antigenic and allergenic cross-reactivity of *Epicoccum nigrum* with other fungi. *Ann Allergy Asthma Immunol.* 89, 285-91.
- Black, P. N., Udy, A. A., Brodie, S. M., 2000. Sensitivity to fungal allergens is a risk factor for life-threatening asthma. *Allergy.* 55, 501-4.
- Chattopadhyay, B. P., Das, S., Adhikari, A., Alam, J., 2007. Exposure to varying concentration of fungal spores in grain storage godowns and its effect on the respiratory function status among the workers. *Ind Health.* 45, 449-61.
- Hamilton, P. B., Lucas, G. B., Welty, R. E., 1969. Mouse toxicity of fungi of tobacco. *Appl Microbiol.* 18, 570-4.
- Karlsson-Borga, A., Jonsson, P., Rolfsen, W., 1989. Specific IgE antibodies to 16 widespread mold genera in patients with suspected mold allergy. *Ann Allergy.* 63, 521-6.
- Koivikko, A., Viander, M., Lanner, A., 1991. Use of the extended Phadebas RAST panel in the diagnosis of mould allergy in asthmatic children. *Allergy.* 46, 85-91.
- Mazur, L. J., Kim, J., 2006. Spectrum of noninfectious health effects from molds. *Pediatrics.* 118, e1909-1926.
- Meng, J., Barnes, C. S., Rosenwasser, L. J., 2012. Identity of the fungal species present in the homes of asthmatic children. *Clin Exp Allergy.* 42, 1448-58.
- Neas, L. M., Dockery, D. W., Burge, H., Koutrakis, P., Speizer, F. E., 1996. Fungus spores, air pollutants, and other determinants of peak expiratory flow rate in children. *Am J Epidemiol.* 143, 797-807.

Niedoszytko, M., Chelminska, M., Jassem, E., Czestochowska, E., 2007. Association between sensitization to *Aureobasidium pullulans* (*Pullularia* sp) and severity of asthma. *Ann Allergy Asthma Immunol.* 98, 153-6.

Noble, J. A., Crow, S. A., Ahearn, D. G., Kuhn, F. A., 1997. Allergic fungal sinusitis in the southeastern USA: involvement of a new agent *Epicoccum nigrum* Ehrenb. ex Schlecht. 1824. *J Med Vet Mycol.* 35, 405-9.

Portnoy, J., Chapman, J., Burge, H., Muilenberg, M., Solomon, W., 1987. *Epicoccum* allergy: skin reaction patterns and spore/mycelium disparities recognized by IgG and IgE ELISA inhibition. *Ann Allergy.* 59, 39-43.

Pritchard, R. C., Muir, D. B., 1987. Black fungi: a survey of dematiaceous hyphomycetes from clinical specimens identified over a five year period in a reference laboratory. *Pathology.* 19, 281-4.

Tarlo, S. M., Fradkin, A., Tobin, R. S., 1988. Skin testing with extracts of fungal species derived from the homes of allergy clinic patients in Toronto, Canada. *Clin Allergy.* 18, 45-52.

Weber, R. W., 2006. On the cover: *Epicoccum pupurascens*. *Ann Allergy Asthma Immunol.* 96, A6.

3.8 Malassezia

3.8.1 Uptake and Systemic Spread

Malassezia species are unique among fungal species in being the only yeast normally present as part of the cutaneous commensal microflora. Studies report that 97% of clinically healthy people have *Malassezia* on their scalp and 92% have it on their trunk; therefore its mere presence is not indicative of disease (Gupta et al., 2001). Under some situations, *Malassezia* species are believed to be opportunistic pathogens in humans. *Malassezia* infection can be systemic in infants and children, or in immunosuppressed adults. However, *Malassezia* systemic infection is not usually the result of spreading from a skin infection, but rather from contamination of intravascular devices or from lipid infusion (Ashbee and Evans, 2002; Gaitanis et al., 2012).

3.8.2 Health Effects in Humans

3.8.2.1 Site of Contact Effects

At the present time, fourteen different species of *Malassezia* (*M. restricta*, *M. obtusa*, *M. slooffiae*, *M. dermatis*, *M. japonica*, *M. nana*, *M. yamatoensis*, *M. equine*, *M. caprae*, *M. cuniculi*, *M. furfur*, *M. sympodoialis*, *M. pachydermatis*, *M. globosa*) have been identified (Gaitanis, et al., 2012) and except for *M. pachydermatis*, the species require an exogenous source of lipid for growth (Ashbee, 2006). *Malassezia* is prevalent in humans as part of the normal

cutaneous microflora, and the presence of *Malassezia* species was confirmed on various anatomical locations of 20 clinically healthy patients, as well as 110 patients with different dermatoses (atopic dermatitis, psoriasis, seborrheic dermatitis, and pityriasis versicolor; Gupta et al., 2001). Interestingly, recovery of *Malassezia* species from skin was significantly lower in patients with the dermatoses than in healthy patients. However, those with the dermatoses excrete skin lipids differing in composition from those of normal skin (Gupta et al., 2001).

The *Malassezia* species dependent on lipids express esterases and lipases that produce fatty acids, lipoxygenases, and proteases that further contribute to symptoms of infection (Cafarchia et al., 2008). Lipid metabolism produces irritant lipid metabolites and highly active indole compounds that bind to specific receptors. The lipases and phospholipases increase free fatty acids that are metabolized by lipoxygenases to bioactive lipid peroxides.

Malassezia is associated with pityriasis versicolor, a skin disease characterized by hypo or hyper-pigmented plaques located in the seborrheic regions of the back, skin, chest, and neck (Ashbee and Evans, 2002). Pityriasis versicolor is associated with *M. globosa* and *M. furfur* (Gaitanis et al., 2012). The production of melanin and a broad array of indole like compounds appear to play a role in the pathogenesis of infections, particularly those associated with pityriasis versicolor (Hort and Mayser, 2011; Gaitanis et al., 2012). Pigment synthesis occurs in *Malassezia* by two pathways: melanin production and formation of tryptophan-derived indole pigments.

Seborrheic dermatitis, a relapsing skin disease of the scalp, eyebrows, paranasal folds, chest, back, axillae, and genitals, is characterized by erythema and scaling. *M. restricta*, *M. globosa*, *M. furfur*, *M. sympodialis* and *M. obtusa* have been associated with seborrheic dermatitis. *M. restricta* and *M. globosa* are the most commonly isolated species. Dandruff, like seborrheic dermatitis, is a similar skin disease of the scalp. *M. globosa* and *M. restricta* are the predominate *Malassezia* species associated with dandruff scalp. *M. globosa* is thought to be the initiating organism by virtue of its high lipase activity (Dawson, 2007). Both seborrheic dermatitis and dandruff are linked to *Malassezia*, but the causal agent is controversial.

Malassezia folliculitis consists of pruritic papules and pustules that occur mainly on the trunk and upper arms but the infection appears to be secondary to follicular occlusion (Ashbee and Evans, 2002). *M. restricta* and *M. globosa* are associated with this skin disease (Gaitanis et al., 2012).

Malassezia infections have also been associated with cases of malignant otitis externa, and onychomycoses (or fungus infection of the nails), that is characterized by thickened and discolored nails. Chowdhary et al. (2005) reports a case in which histopathology was used to identify that the nail infection was due to *M. furfur* (Gaitanis et al., 2012).

Malassezia has been associated with a wide range of other superficial diseases, including acne vulgaris, nodular hair infection, and psoriasis (Ashbee and Evans, 2002). The complexity of

psoriasis pathogenesis and the poor effectiveness of anti-fungal drugs suggest that *Malassezia* plays only a secondary role in psoriasis; possibly that of an exacerbating factor (Gaitanis et al., 2012).

Thus, *Malassezia* infections are associated with a large number of dermal conditions, including commonly occurring conditions, such as seborrheic dermatitis and dandruff. In addition, *Malassezia* is also associated with pityriasis versicolor; a skin disease characterized by pigmented area, and is linked with several diseases of the nails.

3.8.2.2 Irritation

No information was located on the potential for *Malassezia* to cause irritation.

3.8.2.3 Sensitization, Inflammation, Allergy and Asthma

Allergic reactions to fungi, such as *Malassezia*, are associated with increased asthma symptoms and severity, increased asthma risk, and even death. *Malassezia* spores are 2-20 µm in diameter and, thus, are able to descend into the lower respiratory tract and lead to allergic symptoms (Pourfathollah et al., 2014). In a case-control study, *M. pachydermatis* was found on the skin and in the lungs of asthma patients and not the controls; the authors estimated that as many as 20-25% of the asthma patients were infected (van Woerden et al., 2013).

Atopic eczema, a chronic inflammatory disease with unknown etiology, is also associated with a number of *Malassezia* species (Gaitanis et al., 2012). Patients with atopic chronic eczema (an inflammatory relapsing disease) are sensitized to *M. sympodialis* (Gaitanis et al., 2012). *Malassezia* produce a number of allergens that can bind to IgE, altering immune responses (Ashbee and Evans, 2002). IgE-binding allergens were identified in *M. sympodialis*, *M. furfur* and *M. globosa*. The most common species on human skin is *M. globosa* and most atopic eczema patients have IgE reactivity to *M. globosa* antigens. Patients with atopic eczema are also sensitive to allergens from *M. sympodialis*. Healthy control patients or patients with inhalant allergies or urticaria showed no IgE-mediated reaction to the *M. sympodialis*, indicating sensitization to *M. sympodialis* is highly specific for patients with atopic eczema (Casagrande et al., 2006; Gaitanis et al., 2012). When *Malassezia* extracts were tested for inducing sensitivity, with a skin patch test in patients with atopic eczema, 53% of patients had a positive response while only a 2% of control healthy patients tested positive (Casagrande et al., 2006). It is not known why patients with atopic eczema show sensitization against *M. sympodialis* but it may be that these microbes act as a trigger for this disease.

Clearly, *Malassezia* can act as an allergen and sensitize the affected individuals to subsequent *Malassezia* exposure, resulting in enhanced allergic responses. In asthmatic individuals sensitized to *Malassezia*, exposure to the fungus increases the risk, severity and frequency of attacks.

3.8.2.4 Systemic Effects

Systemic disease has only been reported for *M. pachydermatis* and for the lipophilic species, for example *M. furfur* (Ashbee and Evans, 2002). Systemic diseases from *Malassezia* are primarily opportunistic and appear to be increasing, especially in newborns and immunosuppressed patients (Pedrosa et al., 2014). In addition, the majority of these diseases occur in patients with serious health problems or patients using central vascular catheters for the infusion of nutrition, particularly when lipid supplementation is required (Gaitanis et al., 2012). It is particularly difficult to identify specific manifestations of fungemia (fungi in blood) in patients with severe underlying disease; however, neonates can display signs and symptoms of sepsis and thrombocytopenia. *Malassezia* can cause fatal fungemia in premature neonates and, less frequently, in immunocompromised adults (Ashbee and Evans, 2002).

In adults, fever may be the only manifestation and some patients are asymptomatic. The primary clinical manifestations attributed to *Malassezia furfur* sepsis were seen in a female adult who was receiving parenteral nutritional supplements with lipids due to a complicated pregnancy. The clinical manifestations were fever, chills, dyspnea, pleuritic chest pain, and multiple bilateral pulmonary nodular infiltrates (Shparago et al., 1995). In addition, the clinical syndromes reported as a result of *Malassezia* systemic infections in adults include endocardial mass, pneumonia, osteomyelitis, and meningitis (Gaitanis, 2012). *Malassezia* is also reported to cause infections, including mastitis (infection of the breast tissue), septic arthritis, pulmonary vasculitis (inflammation of the blood vessels), and peritonitis (infection of the lining of the abdominal wall) (Ashbee and Evans, 2002; Ashbee, 2006).

3.8.3 Health Effects in Animals

Malassezia pachydermatis is the only non-lipid dependent species of *Malassezia* and is found on the skin surface and in the ear canal of dogs (Cafarchia and Otranto, 2008). Dermatitis in dogs is characterized as pruritic erythematous lesions, usually affecting the abdomen. Otitis in dogs is associated with *Malassezia*, particularly with *M. pachydermatis* (Ashbee, 2007). *Malassezia* has been isolated from the external ear canal and mucosa of healthy cats as well as cats with otitis externa and dermatitis (Crosaz et al., 2013).

3.8.4 References

- Ashbee, H. R., 2006. Recent developments in the immunology and biology of *Malassezia* species. *FEMS Immunol Med Microbiol.* 47, 14-23.
- Ashbee, H. R., 2007. Update on the genus *Malassezia*. *Med Mycol.* 45, 287-303.
- Ashbee, H. R., Evans, E. G., 2002. Immunology of diseases associated with *Malassezia* species. *Clin Microbiol Rev.* 15, 21-57.

- Cafarchia, C., Otranto, D., 2008. The pathogenesis of *Malassezia* yeasts. *Parassitologia*. 50, 65-67.
- Casagrande, B. F., Flückiger, S., Linder, M. T., Johansson, C., Scheynius, A., Cramer, R., Schmid-Grendelmeier, P., 2006. Sensitization to the yeast *Malassezia sympodialis* is specific for extrinsic and intrinsic atopic eczema. *J Invest Dermatol*. 126, 2414-2421.
- Chowdhary, A., Randhawa, H. S., Sharma, S., Brandt, M. E., Kumar, S., 2005. *Malassezia furfur* in a case of onychomycosis: Colonizer or etiologic agent? *Med Mycol*. 43, 87-90.
- Crosaz, O., Legras, A., Vilaplana-Grosso, F., Debeaupuits, J., Chermette, R., Hubert, B., Guillot, J., 2013. Generalized dermatitis associated with *Malassezia* overgrowth in cats: A report of six cases in France. *Med Mycol Case Rep*. 13, 59-62.
- Dawson, T. L., Jr., 2007. *Malassezia globosa* and *restricta*: breakthrough understanding of the etiology and treatment of dandruff and seborrheic dermatitis through whole-genome analysis. *J Invest Dermatol Symp Proc*. 12, 15-19.
- Dankner, W.M., Spector, S.A., Fierer, J., Davis, C.E. 1987. *Malassezia fungemia* in neonates and adults: complication of hyperalimentation. *Rev Infect Dis*. 9(4), 743-753. (Abstract only).
- Gaitanis, G., Magiatis, P., Hantschke, M., Bassukas, I. D., Velegraki, A., 2012. The *Malassezia* genus in skin and systemic diseases. *Clin Microbiol Rev*. 25, 106-141.
- Gupta, A. K., Kohli, Y., Summerbell, R. C., Faergemann, J., 2001. Quantitative culture of *Malassezia* species from different body sites of individuals with or without dermatoses. *Med Mycol*. 39, 243-251.
- Hort, W., Mayser, P., 2011. *Malassezia* virulence determinants. *Curr Opin Infect Dis*. 24, 100-105.
- Pedrosa, A. F., Lisboa, C., Goncalves Rodrigues, A., 2014. *Malassezia* infections: a medical conundrum. *J Am Acad Dermatol*. 71, 170-176.
- Pourfathollah, A. A., Beyzayi, F., Khodadadi, A., Athari, S. S., 2014. General overview of fungal allergic asthma. *J Mycol Res*. 1, 35-41.
- Shparago, N. I., Bruno, P. P., Bennett, J., 1995. Systemic *Malassezia furfur* infection in an adult receiving total parenteral nutrition. *J Am Osteopath Assoc*. 95, 375-377. (Abstract only)
- Van Woerden, H. C., Gregory, C., Brown, R., Marchesi, J. R., Hoogendoorn, B., Matthews, I. P., 2013. Differences in fungi present in induced sputum samples from asthma patients and non-atopic controls: a community based case control study. *BMC Infect Dis*. 13.

3.9 Penicillium

Penicillium species are comprised of teleomorphic genera (*Eupenicillium* and *Talaromyces*) and anamorphic *Penicillium* (Andrianopoulos, 2002). Although most spread via filamentous growth and asexual development, some species, specifically *P. marneffe*, are dimorphic and grow as a monocellular yeast (Andrianopoulos, 2002). (See Volume 1 for more information on these terms and mold biology.)

3.9.1 Uptake and Systemic Spread

Although inhaled *Penicillium* spores are deposited in the alveolar region at a high rate, there is a low potential for causing human infection. *Penicillium* growth is almost completely inhibited at normal body temperatures (37° C) (Schinabeck and Ghannoum, 2003; Versalovic et al., 2011). However, there is evidence that some species, specifically *P. marneffe*, can survive normally functioning immune systems until *in vivo* conditions favor growth (e.g., immunosuppression), up to 11 years (Cooper and Vanittanakom, 2008). Isolation of *Penicillium* species from lesions is not necessarily indicative of etiology, unless there is evidence of growth (e.g., typical fungal elements found in tissues or exudates) (Versalovic et al., 2011).

3.9.2 Health Effects in Humans

Due to the limited pathogenic nature of *Penicillia*, the major cause of human health effects is the production of mycotoxins (EPA, 1997). Hundreds of mycotoxins are associated with *Penicillium* species (EPA, 2004). Of these, citrinin and ochratoxin are of specific interest, and are addressed in Sections 4.5 and 4.6, respectively. Other mycotoxins include roquefortine, isofumigaclavin C, penicillic acid, PR toxin, patulin, and botryodiplodin (EPA, 1997). These mycotoxins are associated with liver, kidney, and nerve damage, mutagenesis and tumorigenesis (EPA, 1997). It is possible that even strains of *P. roqueforti*, which is commonly found in blue cheese, can produce mycotoxins (EPA, 1997). However, *Penicillium* species themselves are infrequently associated with disease (Cooper and Vanittanakom, 2008).

3.9.2.1 Site of Contact Effects

Most reported infections (approximately 60% of cases) are superficial lesions, specifically keratitis, onychomycosis (infection of the nails), otomycosis (ear infection), or cutaneous penicilliosis (López-Martínez et al., 1999; Lyratzopoulos et al., 2002; Versalovic et al., 2011). Although rare, especially in immunocompetent persons, *Penicillium* species other than *P. marneffe* have been isolated from otherwise healthy individuals (Lopez-Martinez et al., 1999; Lyratzopoulos et al., 2002).

3.9.2.2 Irritation

No information was located on the potential for *Penicillium* to cause irritation.

3.9.2.3 Sensitization, Inflammation, Allergy, and Asthma

Penicillium species are known to cause allergy, asthma and hypersensitivity (Shen and Han, 1998; Versalovic et al., 2011). Many molds, including *Penicillium*, produce allergens that are found in germinating spores, hyphal tips, and mycelia (HUD, 2006). Inhaled spores are deposited in the alveolar region at a relatively high rate due to their small size of the spores (approximately 3 microns) (Mazur and Kim, 2006). The fungi colonize in the respiratory tract, resulting in the potential for IgE- (type I) and IgG- (type III) mediated hypersensitivity reactions (Mazur and Kim, 2006), as well as immediate and delayed asthmatic responses in sensitized individuals (Horner et al., 1995).

Penicillium species are associated with skin reactivity and sensitivity among asthmatic patients, including children (Karlsson-Borga et al., 1989; Niedostrytko et al., 2007; Seltzer and Fedoruk, 2007), although these reactions are smaller in intensity and quantity when compared to other molds (e.g., *Cladosporium*, *Alternaria*, and *Fusarium*) (Tarlo et al., 1988). Positive bronchial challenges to *Penicillium* spores, which produce symptoms of asthma, and positive skin-prick test results indicate a causal link between *Penicillium* exposure and asthma (IOM, 2000; Mazur and Kim, 2006). However, dermal sensitivity to *Penicillium* was not associated with an increase in asthma severity or exacerbations requiring hospitalization (Niedostrytko et al., 2007).

Penicillium-mediated allergy and asthma is not limited to adults. *Penicillium* exposure in the home has been linked to increased risk for developing both allergic symptoms and asthma among infants (1-12 months of age) and children, although the increased risk is also associated with other mold contaminations (e.g., *Cladosporium*) (Gent et al., 2002; Meng et al., 2012; Portnoy et al., 2005).

In addition to asthma, *Penicillium* exposure has been linked to other hypersensitivity disorders, including hypersensitivity pneumonitis (HP) (Mazur et al., 2006; do Pico 1976), allergic alveolitis (Schwab and Straus, 2004), and allergic bronchopulmonary aspergillosis (ABPA) (Mazur and Kim, 2006), a disease characterized by eosinophilia, bronchial widening and obstruction, moderate-to-severe asthma, and other tuberculosis-like symptoms (EPA, 2004; Kilch, 2009; Mazur and Kim, 2006). Although *Penicillium* has been associated with ABPA, other molds, specifically *Aspergillus*, are more commonly the etiologic agent (Mazur and Kim, 2006).

One case of occupational exposure to *P. roqueforti* resulted in sensitization. A man who manufactured bleu cheese developed cough, dyspnea, malaise, reduced lung volume, and bibasilar crackles, or crackles originating at the base of the lung (EPA, 1997). Bronchoalveolar lavage fluid and serum contained lymphocytes and antibodies against *P. roqueforti*, indicating that it is linked to the observed symptoms (EPA, 1997).

Overall, *Penicillium* exposure is associated with allergy, asthma, and hypersensitivity in both adults and children.

3.9.2.4 Systemic Effects

Although *Penicillium* species are opportunistic pathogens, their pathogenic potential in immunocompetent persons is very low (EPA, 1997). However, *Penicillia* have been associated with invasive infections (hyalohyphomycoses), specifically endophthalmitis, an infection inside the eyeball (EPA, 1997), peritonitis, urinary tract or kidney infection, endocarditis, sinus and lung infection, and fungemia (Lyratzopoulos et al., 2002; Schinabeck and Ghannoum, 2003; Versalovic et al., 2011). *Penicillia* can also be found in the blood or bone marrow (Versalovic et al., 2011). One case study isolated *P. chrysogenum* from a brain abscess in an immunocompetent person, but there is no information regarding how this infection began (Lyratzopoulos et al., 2002). One other immunocompetent patient developed a *P. decumbens* infection in the paravertebral soft tissue following surgery (Lyratzopoulos et al., 2002). Most invasive infections are usually disseminated across multiple organs (Versalovic et al., 2011). *Penicillium* species have also been reported to proliferate within host phagocytic cells, resulting in granulomas in immunodeficient persons (Versalovic et al., 2011).

Penicillium are more likely to cause opportunistic invasive fungal infections in immunocompromised persons, specifically AIDS patients (Ascioglu et al., 2002). However the risk of infections caused by species other than *P. marneffei* is low; only four cases have been reported of AIDS patients with penicilliosis, or *Penicillium* infection, caused by non-*P. marneffei* species (Cooper and Vanittanakom, 2008). With the exception of *P. marneffei*, *Penicillium* species found in the blood are generally an indication of specimen contamination and not proof of systemic infection (Ascioglu et al., 2002). *P. marneffei*, however, is a significant pathogenic risk to AIDS patients in Southeast Asia, possibly, in part, due to its dimorphic nature (Cooper and Vanittanakom, 2008; Duong, 1996; Marques et al., 2000; Schinabeck and Ghannoum, 2003). Infections caused by *P. marneffei* are called “penicilliosis marneffei” (Cooper and Vanittanakom, 2008) and can be isolated to the point of infection (focal) or disseminate throughout the body (Schinabeck and Ghannoum, 2003). Some cases of penicilliosis marneffei (approximately 20%) have also been reported in immunocompetent persons (Cooper and Vanittanakom, 2008; Duong, 1996; Schinabeck and Ghannoum, 2003).

In general, *Penicillia* are unlikely to be pathogenic, however, they are an opportunistic pathogen and can cause invasive, systemic infections in both immunocompetent and immunocompromised persons. *P. marneffei* appears to pose the greatest risk to immunocompromised individuals.

3.9.3 Health Effects in Animals

Anecdotal reports link *P. roqueforti* with spontaneous abortions in cattle that ate contaminated feed (EPA, 1997), although this information is unreliable. Scientific testing using Koch’s

postulates has not confirmed an effect, and the effect is likely not relevant to human health. It is likely that these effects were observed due to mycotoxins in the feed. In general, *P. roqueforti* is not a known pathogen in animals (EPA, 1997).

One study reported some systemic toxicity or effects following ip injection of *Penicillium* cultures isolated from cured and noncured tobacco into mice (Hamilton et al., 1969); however ip injection is not an environmentally relevant route.

3.9.4 References

Andrianopoulos, A., 2002. Control of morphogenesis in the human fungal pathogen *Penicillium marneffeii*. Int J Med Microbiol. 292, 331-347.

Ascioglu, S., Rex, J. H., de Pauw, B., Bennett, J. E., Bille, J., Crokaert, F., Denning, D. W., Donnelly, J. P., Edwards, J. E., Erjavec, Z., Fiere, D., Lortholary, O., Maertens, J., Meis, J. F., Patterson, T. F., Ritter, J., Selleslag, D., Shah, P. M., Stevens, D. A., Walsh, T. J., 2002. Defining opportunistic invasive fungal infections in immunocompromised patients with cancer and hematopoietic stem cell transplants: An international consensus. Clin Infect Dis. 34, 7–14.

Cooper, C. R., Vanittanakom, N., 2008. Insights into the pathogenicity of *Penicillium marneffeii*. Future Microbiol. 3, 43-55.

Duong, T. A., 1996. Infection due to *Penicillium marneffeii*, an emerging pathogen: Review of 155 reported cases. Clin Infect Dis. 23, 125-130.

EPA (U.S. Environmental Protection Agency), 2004. Guidance for clinicians on the recognition and management of health effects related to mold exposure and moisture indoors. United States Environmental Protection Agency.

EPA, (U.S. Environmental Protection Agency), 1997. *Penicillium roqueforti* Final Risk Assessment. Biotechnology Program under the Toxic Substance Control Act (TSCA). United States Environmental Protection Agency.

Gent, J. F., Ren, P., Belanger, K., Triche, E., Bracken, M. B., Holford, T. R., Leaderer, B. P., 2002. Levels of household mold associated with respiratory symptoms in the first year of life in a cohort at risk for asthma. Environ Health Perspect. 110, 781-786.

Hamilton, P. B., Lucas, G. B., Welty, R. E., 1969. Mouse toxicity of fungi of tobacco. Appl Microbiol. 18, 570-574.

Horner, W. E., Helbling, A., Salvaggio, J. E., Lehrer S B, 1995. Fungal Allergens. Clin Microbiol Rev. 8, 161–179.

HUD, 2006. Healthy Homes Issues: Mold. U.S. Department of Housing and Urban Development.

- IOM, 2000. Clearing the air: asthma and indoor air exposures. Institute of Medicine, National Academy Press, Washington, D.C.
- Karlsson-Borga, A., Jonsson, P., Rolfsen, W., 1989. Specific IgE antibodies to 16 widespread mold genera in patients with suspected mold allergy. *Ann Allergy*. 63, 521-526.
- Klich, M. A., 2009. Health effects of *Aspergillus* in food and air. *Toxicol Ind Health*. 25, 657-667.
- López-Martínez, R., Neumann, L., González-Mendoza, A., 1999. Case Report: Cutaneous penicilliosis due to *Penicillium chrysogenum*. *Mycoses*. 42, 347–349.
- Lyratzopoulos, G., Ellis, M., Nerringer, R., Denning, D. W., 2002. Invasive infection due to penicillium species other than *P. marneffei*. *J Infect*. 45, 184-195.
- Marques, S. A., Robles, A. M., Tortorano, A. M., Tuculet, M. A., Negroni, R., Mendes, R. P., 2000. Mycoses associated with AIDS in the Third World. *Med Mycol*. 38, 269-279.
- Mazur, L. J., Kim, J., [Committee on Environmental Health, American Academy of Pediatrics](#), 2006. Spectrum of noninfectious health effects from molds. *Pediatrics*. 118, e1909-1926.
- Meng, J., Barnes, C. S., Rosenwasser, L. J., 2012. Identity of the fungal species present in the homes of asthmatic children. *Clin Exp Allergy*. 42, 1448-1458.
- Niedoszytko, M., Chelminska, M., Jassem, E., Czestochowska, E., 2007. Association between sensitization to *Aureobasidium pullulans* (*Pullularia sp*) and severity of asthma. *Ann Allergy Asthma Immunol*. 98, 153-156.
- Portnoy, J. M., Kwak, K., Dowling, P., VanOsdol, T., Barnes, C., 2005. Health effects of indoor fungi. *Ann Allergy Asthma Immunol*. 94, 313-319.
- Schinabeck, M. K., Ghannoum, M. A., 2003. Human hyalohyphomycoses: a review of human infections due to *Acremonium* spp., *Paecilomyces* spp., *Penicillium* spp., and *Scopulariopsis* spp. *J Chemother*. 15 Suppl 2, 5-15.
- Schwab, C. J., Straus, D. C., 2004. The roles of *Penicillium* and *Aspergillus* in sick building syndrome. *Adv Appl Microbiol*. 55, 215-238.
- Seltzer, J. M., Fedoruk, M. J., 2007. Health effects of mold in children. *Pediatr Clin N Am*. 54, 309-333.
- Shen, H. D., Han, S. H., 1998. Characterization of allergens of *Penicillium* and *Aspergillus* species. *J Microbiol Immunol Infect*. 31, 141-5.
- Tarlo, S. M., Fradkin, A., Tobin, R. S., 1988. Skin testing with extracts of fungal species derived

from the homes of allergy clinic patients in Toronto, Canada. Clin Allergy. 18, 45-52.

Versalovic, J., Carroll, K. C., Funke, G., Jorgensen, J. H., Landry, M. L., Warnock, D. W. Eds.), 2011. Manual of Clinical Microbiology. 10th Edition, Vol. 2. . ASM Press, Washington, D.C.

3.10 Phoma

3.10.1 Uptake and Systemic Spread

No information was located on the potential for *Phoma* species to cause systemic effects or produce a toxin. Most reported effects occur at the site of contact (e.g., skin or lung). Only one reported case was located indicating the potential for *Phoma* infections to spread and cause serious systemic infection. This was a case report of an infection in an immunocompromised neonate that progressed from rhinosinusitis to rhinocerebral infection (infection of the sinuses, nasal passages, oral cavity, and brain), ultimately resulting in death (Roehm et al., 2012).

3.10.2 Health Effects in Humans

3.10.2.1 Site of Contact Effects

Phoma species are associated with a variety of local skin infections and lesions.

Immunocompromised or suppressed persons are most commonly infected (Baker et al., 1987; Bakerspigel, 1970; Dooley et al., 1989; Everett et al., 2003; Oh et al., 1999; Rosen et al., 1996; Shukla et al., 1984; Young et al., 1973; Zaitz et al., 1997), but infections are also noted in healthy, or immunocompetent, people as well (Bakerspigel et al., 1981; Gordon et al., 1975; Hirsh and Schiff, 1996; Isa-Isa et al., 2012; Rai, 1989; Stone et al., 1988; Vasoo et al., 2011). These infections may produce erythematous (red) (Bakerspigel et al., 1981; Rai, 1989; Rosen et al., 1996; Shukla et al., 1984; Zaitz et al., 1997) and scaly lesions (Arrese et al., 1997; Shukla et al., 1984), which may be exacerbated in persons taking corticosteroids (Rosen et al., 1996; Shukla et al., 1984). *Phoma* has also been identified as an etiologic agent in Majocchi's granuloma (MG), a dermatophytic infection, in both healthy and immunocompromised persons (Ilkit et al., 2012). *Phoma* has also been associated with cases of onychomycosis (Tullio et al., 2010), keratitis (Rishi and Font, 2003), and other superficial skin infections that can be caused by multiple species of fungi (Chan et al., 2013), but the etiological role of *Phoma* in these infections is unclear.

Infections of *Phoma* have been reported on the skin (Everett et al., 2003) and in the respiratory tracts of immunocompromised persons. One case of infection in an neonate with congenital acute lymphoblastic leukemia specifically progressed from rhinosinusitis to rhinocerebral infection resulting in death (Roehm et al., 2012). Another case of lung infection was found in a 68 year old male with acute myeloid leukemia (Balis et al., 2006). The man died from cardiopulmonary arrest after surgery to remove the fungal mass from his lung.

Phoma may be associated with interstitial pneumonitis (Green, 1972). Patients with suspected pneumonitis or aspergillosis were found to have a positive antibody reaction to *Phoma* (Green, 1972). Although the *Phoma* used for the testing came from a shower curtain, the investigators did not further evaluate whether the patients had *Phoma* exposure, or whether the response reflected cross-reactivity.

Overall, *Phoma* species are associated with superficial and invasive infections of the skin and lungs, particularly in people with compromised immune systems, but specific dose-response information has not been identified. While some members are pathogenic, those members are generally considered to be of less concern compared to other pathogenic molds (Pritchard and Muir, 1987).

3.10.2.2 Irritation

Symptoms of irritation were reported by workers in a building contaminated (known moisture problems for 10 years) with *Trichoderma*, *Phoma*, and *Fusarium* (Ebbehoj et al., 2002). Prior to remediation, a high percentage of the 25 employees evaluated complained of symptoms, such as irritated eyes (88%), irritated throat (84%), and fatigue (88%), with fewer reporting nose irritation (22%) or dermal rash (36%). The causal relationship between symptoms and exposure (particularly *Phoma* exposure) is unclear, particularly in light of the exposure to multiple molds, but symptoms improved upon eradication of the molds.

Overall, *Phoma* species are associated, although rarely causally linked, with a number of reports of irritation and inflammation of the eyes, skin, and respiratory tract. The etiologic contribution of *Phoma* is uncertain, given symptoms of irritation are elicited from multiple mold exposures, and no dose-response information was identified.

3.10.2.3 Sensitization, Inflammation, Allergy and Asthma

Epidemiology studies have identified an increase in allergic rhinitis, asthma, and asthma-like symptoms (e.g., wheezing and long-term cough) among children exposed to home or school environments with moisture problems (Tarlo et al., 1988; Taskinen et al., 1997). Positive reactions to skin-prick tests (Szantho et al., 1992; Tarlo et al., 1988; Taskinen et al., 1997) and the Phadebas RASTTM technique (Karlsson-Borga et al., 1989; Koivikko et al., 1991) indicate that some of these asthma cases may be caused by *Phoma*, although there is a high potential for cross-reactivity among mold species and multiple mold sensitivity. *Phoma* has also been linked to asthma in adults. Of 14 patients with allergic rhinitis or asthma, 5 (36%) had positive skin-prick test reactions to *Phoma glomerata* (Tarlo et al., 1988). Additionally, respiratory decrements were reported by adults employed in a building contaminated with *Trichoderma*, *Phoma*, and *Fusarium* (Ebbehoj et al., 2002). Exposure to these molds increased the peak-expiratory flow (PEF) variability, where PEF is a measurement of the maximum air flow exhaled with maximal force immediately following a full inspiration. Asthmatics have greater variability in their daily

PEF measurements when compared to healthy individuals. The causal relationship between symptoms and exposure is unclear, but variability decreased upon eradication of the molds. Overall, *Phoma* species may contribute to new-onset or exacerbation of asthma, but the causal link is neither clear nor quantifiable.

Phoma is also linked to cases of other respiratory illnesses, such as hypersensitivity pneumonitis (HP), or allergy-related lung inflammation, resulting from exposure to mold growth in a saxophone and use of a contaminated humidifier (Metzger et al., 2010; Moran et al., 2002). The causal link between HP and *Phoma* is unclear since all reported cases were also exposed to at least one other mold as well.

Current data suggests that allergic rhinoconjunctivitis and atopic dermatitis in children are generally not associated with moisture or mold exposure (Taskinen et al., 1997).

Overall, *Phoma* species are associated, although rarely causally linked, with a number of reports of allergy and asthma-like symptoms. The etiologic contribution of *Phoma* is uncertain, considering exposure to and cross-reactivity with other isolated molds, and no dose-response information was identified. *Phoma* is directly linked, however, with cases of HP.

3.10.2.4 Systemic Effects

Fatigue and dizziness have been reported among people employed in a building contaminated with *Trichoderma*, *Phoma*, and *Fusarium* (Ebbehoj et al., 2002). The causal relationship between the symptoms and *Phoma* exposure is unclear, in light of the exposure to other molds, but symptoms improved upon eradication of the molds.

A *Phoma* infection within the respiratory tract progressed to rhinocerebral infection and death in an neonate with congenital acute lymphoblastic leukemia (Roehm et al., 2012), indicating that *Phoma* infections can become systemic, although the occurrence is unlikely and may only be a concern for immunocompromised persons. The mechanism for the systemic spread of this infection is unknown.

3.10.3 Health Effects in Animals

Animal data support the epidemiological research and case studies that indicate a potential for *Phoma* to cause superficial infections on the skin. Experiments in rabbits show that *Phoma* can cause erythematic skin lesions (Rai, 1989).

Despite the possibility for skin infection, there is no indication from animal data that *Phoma* species cause systemic effects. One study reported a lack of systemic toxicity or effects following ip injection of fungal cultures isolated from cured and noncured tobacco into mice (Hamilton et al., 1969); however ip injection is not an environmentally relevant route.

3.10.4 References

- Arrese, J. E., Pierard-Franchimont, C., Pierard, G. E. 1997. Unusual mould infection of the human stratum corneum. J. Med. Vet. Mycol. 35, 225-227.
- Baker, J. G., Salkin, I. F., Forgacs, P., Haines, J. H., Kemna, M. E. 1987. First report of subcutaneous phaeohyphomycosis of the foot caused by *Phoma minutella*. J. Clin. Microbiol. 25, 2395-2397.
- Bakerspigel, A. 1970. The isolation of *Phoma hibernica* from a lesion on a leg. Sabouraudia 7, 261-264. (Abstract only)
- Bakerspigel, A., Lowe, D., Rostas, A. 1981. The isolation of *Phoma eupyrena* from a human lesion. Arch. Dermatol. 117, 362-363.
- Balis, E., Velegraki, A., Fragou, A., Pefanis, A., Kalabokas, T., Mountokalakis, T. 2006. Lung mass caused by *Phoma exigua*. Scand. J. Infect. Dis. 38, 552-555.
- Chan, G. F., Sinniah, S., Idris, T. I., Puad, M. S., Abd Rahman, A. Z. 2013. Multiple rare opportunistic and pathogenic fungi in persistent foot skin infection. Pak. J. Biol. Sci. 16, 208-218.
- Dooley, D. P., Beckius, M. L., Jeffery, B. S., McAllister, C.K., Radentz, W.H., Feldman, A. R., Rinaldi, M.G., Bailey, S.R., Keeling, J. H. 1989. Phaeohyphomycotic cutaneous disease caused by *Pleurophoma* in a cardiac transplant patient. J. Infect. Dis. 159, 503-507.
- Ebbehoj, N. E., Hansen, M. O., Sigsgaard, T., Larsen, L. 2002. Building-related symptoms and molds: A two-step intervention study. Indoor Air 12, 273-277.
- Everett, J. E., Busick, N. P., Sielaff, T., Wahoff, D. C., Dunn, D. L. 2003. A deeply invasive *Phoma* species infection in a renal transplant recipient. Transplant Proc. 35, 1387-1389.
- Gordon, M. A., Salkin, I. F., Stone, W. B. 1975. *Phoma (Peyronellaea)* as zoopathogen. Sabouraudia 13, 329-333. (Abstract only)
- Green, W. F. 1972. Precipitins against a fungus, *Phoma violacea*, isolated from a mouldy shower curtain in sera from patients with suspected allergic interstitial pneumonitis. Med. J. Aust. 1, 696-698.
- Hamilton, P.B., Lucas, G.B., Welty, R.E. 1969. Mouse toxicity of fungi of tobacco. Appl. Microbiol. 18(4), 570-574.
- Hirsh, A. H., Schiff, T. A. 1996. Subcutaneous phaeohyphomycosis caused by an unusual pathogen: *Phoma* species. J. Am. Acad. Dermatol. 34, 679-680.

- Ilkit, M., Durdu, M., Karakas, M. 2012. Majocchi's granuloma: A symptom complex caused by fungal pathogens. *Med. Mycol.* 50, 449-457.
- Isa-Isa, R., Garcia, C., Isa, M., Arenas, R. 2012. Subcutaneous phaeohyphomycosis (mycotic cyst). *Clin. Dermatol.* 30, 425-431.
- Karlsson-Borga, A., Jonsson, P., Rolfsen, W. 1989. Specific IgE antibodies to 16 widespread mold genera in patients with suspected mold allergy. *Ann. Allergy.* 63, 521-526.
- Koivikko, A., Viander, M., Lanner, A. 1991. Use of the extended Phadebas RAST panel in the diagnosis of mould allergy in asthmatic children. *Allergy* 46, 85-91.
- Metzger, F., Haccuria, A., Reboux, G., Nolard, N., Dalphin, J. C., De Vuyst, P. 2010. Hypersensitivity pneumonitis due to molds in a saxophone player. *Chest* 138, 724-726.
- Moran, J. V., Greenberger, P. A., Patterson, R. 2002. Long-term evaluation of hypersensitivity pneumonitis: A case study follow-up and literature review. *Allergy Asthma Proc.* 23, 265-270.
- Oh, C. K., Kwon, K. S., Lee, J. B., Jang, H. S., Chung, T. A., Suh, S. B. 1999. Subcutaneous phaeohyphomycosis caused by *Phoma* species. *Int. J. Dermatol.* 38, 874-876.
- Pritchard, R. C., Muir, D.B. 1987. Black fungi: a survey of *Dematiaceous hyphomycetes* from clinical specimens identified over a five year period in a reference laboratory. *Pathology* 19(3), 281-284. (Abstract only)
- Rai, M. K. 1989. *Phoma sorghina* infection in human being. *Mycopathologia* 105, 167-170.
- Rishi, K., Font, R. L. 2003. Keratitis caused by an unusual fungus, *Phoma* species. *Cornea* 22, 166-168.
- Roehm, C. E., Salazar, J. C., Hagstrom, N., Valdez, T. A. 2012. *Phoma* and *Acremonium* invasive fungal rhinosinusitis in congenital acute lymphocytic leukemia and literature review. *Int. J. Pediatr. Otorhinolaryngol.* 76, 1387-1391.
- Rosen, T., Rinaldi, M. J., Tschen, J. A., Stern, J. K., Cernoch, P. 1996. Cutaneous lesions due to *Pleurophoma* (*Phoma*) complex. *South Med. J.* 89, 431-433.
- Shukla, N. P., Rajak, R. K., Agarwal, G. P., Gupta, D. K. 1984. *Phoma minutispora* as a human pathogen. *Mykosen* 27, 255-258.
- Stone, M. S., Rosen, T., Clarridge, J. 1988. Phaeohyphomycosis due to *Coelomycetes* organisms. *Int. J. Derm.* 27, 404-405.
- Szantho, A., Osvath, P., Horvath, Z., Novak, E. K., Kujalek, E. 1992. Study of mold allergy in asthmatic children in Hungary. *J. Investig. Allergol. Clin. Immunol.* 2, 84-90.

Tarlo, S. M., Fradkin, A., Tobin, R. S. 1988. Skin testing with extracts of fungal species derived from the homes of allergy clinic patients in Toronto, Canada. *Clin. Allergy*. 18, 45-52.

Taskinen, T., Meklin, T., Nousiainen, M., Husman, T., Nevalainen, A., Korppi, M. 1997. Moisture and mould problems in schools and respiratory manifestations in schoolchildren: clinical and skin test findings. *Acta. Paediatr.* 86, 1181-1187.

Tullio, V., Banche, G., Allizond, V., Roana, J., Mandras, N., Scalas, D., Panzone, M., Cervetti, O., Valle, S., Carlone, N., Cuffini, A. M. 2010. Non-dermatophyte moulds as skin and nail foot mycosis agents: *Phoma herbarum*, *Chaetomium globosum* and *Microascus cinereus*. *Fungal Biol.* 114, 345-349.

Vasoo, S., Yong, L. K., Sultania-Dudani, P., Scorza, M. L., Sekosan, M., Beavis, K. G., Huhn, G. D. 2011. Phaeomycotic cysts caused by *Phoma* species. *Diagn. Microbiol. Infect.Dis.* 70, 531-533.

Young, N. A., Kwon-Chung, K. J., Freeman, J. 1973. Subcutaneous abscess caused by *Phoma* sp. resembling *Pyrenochaeta romeroi*: Unique fungal infection occurring in immunosuppressed recipient of renal allograft. *Am. J. Clin. Pathol.* 59, 810-816.

Zaitz, C., Heins-Vaccari, E. M., de Freitas, R. S., Arriagada, G. L., Ruiz, L., Totoli, S. A., Marques, A. C., Rezze, G. G., Muller, H., Valente, N. S., Lacaz Cda, S. 1997. Subcutaneous phaeohyphomycosis caused by *Phoma* cava. Report of a case and review of the literature. *Rev Inst. Med. Trop. Sao Paulo.* 39, 43-48.

3.11 Stachybotrys

Of the *Stachybotrys* species, information is available primarily on *Stachybotrys chartarum*. The available information indicates that the observed effects are due to the toxins produced by *Stachybotrys*, rather than reflecting an infection (Nielsen, 2003; Kuhn and Ghannoum, 2003), but much of the available data are based on exposure to the whole organism. See Section 4.4 for more information on cyclosporine, and Section 4.8 on other toxins produced by *Stachybotrys*.

3.11.1 Uptake and Systemic Spread

Several authors have considered which portion of the *S. chartarum* organism is responsible for its toxicity. Inflammation and allergic sensitization are observed following airborne exposures, but the relative contributions of airborne spores, hyphae, and fungal fragments to reactions in humans are not well understood (Pestka et al., 2008). The outer plasmalemma (plasma membrane) and the inner wall layers of *Stachybotrys* conidiospores contain macrocyclic trichothecenes, such as satratoxins (see Section 4.8) (Amuzie et al., 2010). The trichothecenes are rapidly released in the rat lung. Fragmented mycelia may also facilitate the delivery of *Stachybotrys* toxins to the respiratory tract. The potentially hemolytic enzyme stachylysin localizes in the inner cell wall of the *S. chartarum* spore (Pestka et al., 2008).

Dry spores are readily aerosolized in a respirable size, and nonviable fine airborne particulates containing satratoxins can be released from cultures (Amuzie et al., 2010). However, spores are not readily aerosolized in the presence of moisture unless the culture is disturbed (EPA, 2004).

Pestka et al. (2008) described several studies evaluating the survival of *S. chartarum* spores following instillation *in vivo*, or in alveolar macrophages *in vitro*. *Stachybotrys* spores were unable to germinate in the lungs of adult rats and were effectively cleared from the lung within 7 days of an acute or short-term repeated exposure. In contrast, the spores were able to germinate in the lungs of 4-day-old rat pups instilled with viable *Stachybotrys* spores. More stachylysin is found in the mouse lung at 72 hours after intratracheal instillation than after 24 hours, suggesting that, in mice, production and/or release is a relatively slow process. *S. chartarum* spores have higher survival in alveolar macrophages (apparently from mice) *in vitro* than do spores from the prototypical fungal respiratory pathogen *Aspergillus fumigatus*, suggesting that *Stachybotrys* is more resistant to removal via phagocytosis in the respiratory tract. Macrophage ingestion may facilitate the inactivation of stachylysin, as has been observed for the hemolysin produced by *Aspergillus fumigatus*.

3.11.2 Health Effects in Humans

Stachybotrys produces a number of toxic metabolites called mycotoxins. The most important among these are trichothecenes (including satratoxins), atronone, stachylysin, hemolysin, proteinases (particularly stachyrase A), glucans, and spirocyclic drimanes (Pestka et al., 2008).

A wide range of symptoms, particularly chronic respiratory symptoms, as well as eye and skin irritation and neurological symptoms, such as fatigue, headaches and dizziness, have been attributed to *Stachybotrys* exposure in numerous reports. Collectively, these symptoms are sometimes referred to as damp building related illnesses (DBRI). Other symptoms reported to be associated with exposure to *Stachybotrys* in contaminated straw include dyspnea, shortness of breath, sore throat, nose bleeds, “burning” ocular pain, periorbital edema, weakness, and exhaustion (EPA, 2004). However, although some studies have shown an association between exposure and health effects, the data are not sufficient to demonstrate a causal relationship (EPA, 2004). This lack of a demonstrated causal relationship has many reasons, including small sample sizes, difficulty in measuring exposure, and difficulty in controlling for other potential causative agents.

3.11.2.1 Site of Contact Effects

Perhaps the endpoint with the strongest support is for generalized respiratory tract symptoms. However, an Institute of Medicine (IOM) report (as cited by Pestka et al., 2008) concluded that there is sufficient evidence for an association between the exposure to moldy, damp buildings and symptoms of the upper and lower respiratory tract, but supportive data are insufficient for other specific health conditions, such as airflow obstruction, mucous membrane irritation,

chronic obstructive pulmonary disease, pulmonary hemorrhage, and neurologic effects. This report also noted that, although animal studies indicate that an association with *Stachybotrys* is plausible, based on *in vitro* and animal studies, the data are insufficient to demonstrate a causal relationship between DBRI and *Stachybotrys*.

One of the more serious and controversial effects attributed to *Stachybotrys* exposure is idiopathic pulmonary hemorrhage/hemosiderosis (IPH). This effect was initially reported in infants living in water-damaged homes in Cleveland, Ohio, and subsequently in Chicago, Illinois, and had a high mortality rate (Nikulin et al., 1997; Chapman et al., 2003; Dearborn et al., 1999). A total of 37 infants were diagnosed as having IPH, with 12 deaths, including 7 originally diagnosed as sudden infant death syndrome (Dearborn et al., 1999). High spore counts of *Stachybotrys* (in the air, 43 colony-forming units [CFU]/m³ vs. 4 CFU/m³ (controls); on surfaces: 20 x 10⁶ CFU/m³ vs. 0.007 x 10⁶ CFU/m³ (controls)) were observed in the homes of affected infants, and the infants had a recurrence of the pulmonary bleeding on return to their homes (Elidemir et al., 1999; Kuhn and Ghannoum, 2003). Other molds including *Aspergillus*, *Cladosporium*, and *Penicillium* were reported to be abundant in the case infants' homes, but matched analyses for these molds failed to demonstrate differences in concentrations between case and control homes (Dearborn et al., 1999). This appeared to implicate *Stachybotrys* as the potential agent in the pathogenesis of IPH in these infants. Although the spores and organisms were not found in the patients in one study, a subsequent unrelated case report isolated *Stachybotrys* from the bronchoalveolar lavage (BAL) fluid of a child with progressive respiratory symptoms and pulmonary hemorrhage, suggesting that the mold is also associated with IPH in older children (Elidemir et al., 1999). Environmental tobacco smoke was frequently present in the infants' homes and has been suggested as a trigger precipitating the acute bleeding (Pestka et al., 2008).

Although the initial findings were supported by the US Centers for Disease Control and Prevention (CDC), two expert CDC panels found serious flaws in the infant studies and concluded that the evidence for *Stachybotrys* as the cause of IPH in the infants was not proven (Chapman et al., 2003; Kuhn and Ghannoum, 2003). Some of the shortcomings that the CDC identified in these cases included: (1) bias in the home samplings (which, if true, could invalidate the studies); (2) *Stachybotrys* being present in a similar number of water-damaged case and control homes, with the water damage not well defined; and (3) sampling being performed weeks to months after exposure (Kuhn and Ghannoum, 2003). Significant differences between case and control infants were also noted with regard to sex, race, birth weight, breastfeeding, smoking, and the presence of electric fans (which may relate to the amount of moisture present), while non-significant differences existed for gestational and maternal age (Kuhn and Ghannoum, 2003). Furthermore, the authors of the cases failed to provide a consistent definition of lung diseases. Since it is not known that the illness was due to a single disease entity, the CDC noted that it is unclear if a single etiology could be responsible (Kuhn and Ghannoum, 2003). In light

of these deficiencies, the data were insufficient to show a causal relationship between *Stachybotrys* and IPH.

Contact with musty straw has been reported to cause dermatitis that progressed to hyperemia (tissue congestion with blood) and crusting exudates, with subsequent resolution. Although the initial symptoms were not definitively attributed to *Stachybotrys*, *S. alternans* isolates from the straw were toxic in a rabbit dermal toxicity test and produced the same local and systemic response when applied to the skin of volunteers (Kuhn and Ghannoum, 2003). The location of lesions on many skin surfaces suggested that the lesions resulted from contact with aerosolized material, rather than direct contact with the organism in the straw. The affected areas (e.g., scrotum, armpit) were regions where the skin is moist and skin-to-skin contact occurs.

3.11.2.2 Irritation

Irritation of the eyes and mucous membranes have been reported as part of DBRI, but causality has not been shown, either for the relationship to damp buildings in general, or *Stachybotrys* in particular (Kuhn and Ghannoum, 2003).

No data were identified indicating that *Stachybotrys* has the potential to be irritating to the skin following exposure. However, fingertip inflammation was reported in three women who handled moldy horticulture pots contaminated with *Stachybotrys conidi* (but not *Stachybotrys chartarum*), *Chaetomilltll perithecia*, and other fungi (Chapman et al., 2003). A mycotoxin was postulated to be the cause, but the etiologic agent or the mechanism (allergic or irritant contact dermatitis, toxicity, or infection) could not be determined because tests were not performed (Chapman et al., 2003).

3.11.2.3 Sensitization, Inflammation, Allergy and Asthma

No clear association has been identified between *Stachybotrys* exposure and allergy/asthma, although some human serum studies have identified IgE specific for *Stachybotrys* antigens, suggesting that *Stachybotrys* can induce sensitization. An association of *Stachybotrys* with allergic symptoms was reported in persons exhibiting DBRI (Cooley et al., 1998). However, other investigations of *Stachybotrys*-contaminated buildings did not find a significant difference between the presence of IgE or IgG antibodies to *Stachybotrys* in case and control individuals (Johanning et al., 1996) or a relationship of these antibodies to the presence of human disease (Hodgson et al., 1998). Pestka et al. (2008) also reported functional IgE specific to *Stachybotrys* in DBRI patients without Type I allergic disease, indicative of sensitization. However, a subsequent study by Lander et al. (2001) observed a significant association between a positive histamine release test (HRT) (*Stachybotrys* specific) and DBRI but not between positive HRT and self-reported hay fever or asthma (Lander et al., 2001). These studies indicate that some people have developed antibodies that are reactive with *Stachybotrys* proteins, but it is not always clear whether these antibodies are a result of direct exposure to *Stachybotrys* or of cross-

reactivity among mold antigens (Pestka et al., 2008). These authors have also noted that the presence of *Stachybotrys* reactive IgE does not always indicate the presence of allergic disease. Instead, it indicates the potential for exposure to trigger an allergic event without prior exposure to *Stachybotrys* due to cross reactivity with another allergen. Other investigators noted that even humans who become ill with DBRI after exposure to *Stachybotrys* often do not develop IgG or IgE anti-*Stachybotrys* antibodies (Dearborn et al., 1999).

Cases of hypersensitivity pneumonitis caused by *Stachybotrys* have not been reported (Chapman et al., 2003).

Overall, the available epidemiological investigations have not yet demonstrated a clear association between *Stachybotrys* exposure and allergy/asthma.

3.11.2.4 Systemic Effects

The systemic effects observed after *Stachybotrys* exposure are attributed to its mycotoxins rather than an effect of the organism itself.

Kuhn and Ghannoum (2003) reviewed the available data on the potential of *Stachybotrys* to cause neurotoxicity and concluded that proof of this health effect is lacking. They noted that much of the support for the association was based on the observation of equine stachybotryotoxicosis, which was characterized by a range of neurologic effects including areflexia (absence of neurological reflexes), hyperirritability, and blindness. The authors also noted that there are many reports of subjective complaints of neurologic symptoms, but no objective evidence has been produced to indicate neurological effects resulting from exposure to *Stachybotrys*.

Recurring cold and flu symptoms, sore throats, diarrhea, headaches, fatigue, dermatitis, intermittent focal alopecia, and generalized malaise, alleged to be caused by inhalation of *Stachybotrys*, were reported in five members of a family and their maid (reviewed in Chapman et al., 2003). However, the results of repeated medical examinations were negative for disease. Epidemiologic studies involving workers in several office buildings that had sustained water damage also reported numerous health complaints, especially neuropsychological and upper respiratory tract symptoms. The symptoms were attributed to fungi, including *Stachybotrys*, which were recovered on bulk samples of water-damaged materials. However, only trace quantities of *Stachybotrys* spores were recovered from air samples (Chapman et al., 2003), indicating that the mold is not likely to be causative agent.

Overall, limited information is available regarding the systemic effects caused by *Stachybotrys*, and the effects observed are likely caused by the toxins produced by *Stachybotrys*.

No information was located indicating that immunocompromised people are a sensitive population. This is consistent with the effects of *Stachybotrys* being due to its toxins rather than a direct effect of the organism.

3.11.3 Health Effects in Animals

Although *Stachybotrys* is not considered to be an infectious agent, there have been reports of limited opportunistic outgrowth following intratracheal instillation in extremely young or immunosuppressed animals, conditions that might exacerbate the effects of inhaled spores (Pestka et al., 2008). *Stachybotrys* did not establish an infection in the rat pups in spite of the germination and outgrowth (Yike and Dearborn, 2004), but the viable spores were more injurious to rat pups than nonviable spores. The available studies seem to suggest that *Stachybotrys* may be noninfectious, but limited opportunistic outgrowth and release of bioactive products (that is, toxins) in extremely young or immunosuppressed animals might exacerbate the effects of inhaled spores (Pestka et al., 2008; Yike and Dearborn, 2004). Yike and Dearborn (2004) noted that stachylysin may be released only with germination of the *Stachybotrys* spores, and this is likely to potentiate the effects of inhaled spores.

Single intranasal instillation of 10^6 *Stachybotrys* spores (isolates that produced satratoxin G and H) to 5-week old adult mice resulted in death of 2 of 4 (50%) of the mice. The surviving mice lost 17% of their weight within 3 days. Severe alveolar, bronchiolar, and interstitial inflammation (neutrophils, macrophages, lymphocytes) with luminal hemorrhagic exudates were observed. Biweekly intranasal administration of 10^5 or 10^3 *Stachybotrys* spores for 3 weeks produced similar dose-dependent inflammation. In both the acute and subacute exposures, instillation of the same number of spores from a strain that did not produce detectable levels of mycotoxins produced a much milder inflammation (Nikulin et al., 1997; Dearborn et al., 1999), indicating that the toxins, and not the *Stachybotrys* spores themselves, are the likely cause of the observed inflammation.

3.11.4 References

- Amuzie, C.J., Islam, Z., Kim, J.K., Seo, J.H., Pestka, J.J. 2010. Kinetics of satratoxin G tissue distribution and excretion following intranasal exposure in the mouse. *Toxicol Sci.* 116(2), 433-440.
- Chapman, J.A., Terr, A.I., Jacobs, R.L., Charlesworth, E.N., Bardana, E.L. Jr., 2003. Toxic mold: phantom risk vs. science. *Annal Allerg Asthma Immunol.* 91, 222-232
- Cooley, J.D., Wong, W.C., Jumper, C.A., Straus, D.C. 1998. Correlation between the prevalence of certain fungi and sick building syndrome. *Occup Environ Med.* 55, 579-584.

- Dearborn, D.G., Yike, I., Sorenson, W.G., Miller, M.J., Etzel, R.A. 1999. Overview of investigations into pulmonary hemorrhage among infants in Cleveland, Ohio. *Environ Health Perspect.* 107(Suppl. 3), 495-499.
- Elidemir, O., Colasurdo, G.N., Rossman, S.N., Fan, L.L. 1999. Isolation of *Stachybotrys* from the lung of a child with pulmonary hemosiderosis. *Pediatrics.* 104, 964-966.
- EPA (U.S. Environmental Protection Agency). 2004. Guidance for Clinicians on the Recognition and Management of Health Effects related to Mold Exposures and Moisture Indoors. Center for Indoor Environments and Health, University of Connecticut. U.S. Environmental Protection Agency.
- Hodgson, M.J., Morey, P., Leung, W.Y., Morrow, L., Miller, D., Jarvis, B.B., Robbins, H., Halsey, J.F., Storey, E. 1998. Building-associated pulmonary disease from exposure to *Stachybotrys chartarum* and *Aspergillus versicolor*. *J Occup Environ Med.* 40(3), 241-249.
- Johanning, E., Biagini, R., Hull, D., Morey, P., Jarvis, B., Landsbergis, P. 1996. Health and immunology study following exposure to toxigenic fungi (*Stachybotrys chartarum*) in a water-damaged office environment. *Int Arch Occup Environ Health.* 68, 207-218.
- Kuhn, D.M., Ghannoum, M.A. 2003. Indoor mold, toxigenic fungi, and *Stachybotrys chartarum*: Infectious disease perspective. *Clin Microbiol Rev.* 16(1), 144-172.
- Lander, F., Meyer, H.W., Norn, S. 2001. Serum IgE specific to indoor moulds, measured by basophil histamine release, is associated with building-related symptoms in damp buildings.
- Leino, M., Makela, M., Reijula, K., Haahtela, T., Mussalo-Rauhamaa, H., Tuomi, T., Hintikka, E.L., Alenius, H. 2003. Intranasal exposure to a damp building mould, *Stachybotrys chartarum*, induces lung inflammation in mice by satratoxin-independent mechanisms. *Clin Exp Allergy.* 33, 1603-1610.
- Nielsen, K.F. 2003. Mycotoxin production by indoor molds. *Fungal Genet Biol.* 39, 103-117.
- Nikulin, M., Reijula, K., Jarvis, B.B., Hintikka, E.L. 1996. Experimental lung mycotoxicosis in mice induced by *Stachybotrys atra*. *Int J Exp Path.* 77, 213-218.
- Nikulin, M., Reijula, K., Jarvis, B.B., Veijalainen, P., Hintikka, E.L. 1997. Effects of intranasal exposure to spores of *Stachybotrys atra* in mice. *Fundam Appl Toxicol.* 35, 182-188.
- Pestka, J.J., Yike, I., Dearborn, D.G., Ward, M.D.W., Harkema, J.R. 2008. *Stachybotrys chartarum*, trichothecene mycotoxins, and damp building-related illness: New insights into a public health enigma. *Toxicol Sci.* 104(1), 4-26.

Yike, I., Dearborn, D.G. 2004. Pulmonary effects of *Stachybotrys chartarum* in animal studies. *Adv Appl Microbiol.* 55, 241-273.

3.12 Zygomycetes

3.12.1 Uptake and Systemic Spread

The usual exposure routes to Zygomycetes are via the respiratory tract, the skin and sometimes the gut (Mantadakis and Samonis 2009). Zygomycoses (i.e., infections by Zygomycetes) that result in systemic spread are considered to be “exceptionally severe” with a high mortality rate (over 50%) due to the capability of the fungi to invade blood vessels (angioinvasion) and spread (Muszenwska et al., 2014). Roden et al. (2005) reported systemic spread in 23% of documented Zygomycosis cases, and of the systemic disease cases mortality was 96%. The invading hyphae of the mold require aggressive therapy of surgical removal and antifungal therapy (Ibrahim et al., 2012).

3.12.2 Health Effects in Humans

Zygomycosis is a “catch all term” that describes a variety of infections caused by members of the Zygomycetes. The term has been used widely in literature, and according to Mantadakis and Samonis (2009), improvements are being made to better evaluate the association between disease and the causative agents. Zygomycetes is the name of the taxonomic class (see microbial section for a review on taxonomy) that includes several genera that can be pathogenic in humans. This review is focused on the most prominent and well-studied/characterized genera associated with human disease (rhinocerebral, pulmonary, allergic reaction, cutaneous effects): *Rhizopus* and *Mucor* (Ribes et al. 2000). Other pathogenic genera with some data include *Basidiobolus* and *Conidiobolus* causing chronic sinusitis, both of which have been isolated from tropical and sub-tropical environmental sources.

Entomophthorales (genera are *Conidiobolus* and *Basiobolus*) are associated with chronic cutaneous and subcutaneous infections that are nearly exclusively limited to the tropics with unlikely dissemination to the internal organs. These diseases should be referred to as “entomophthoramycosis”. Diseases caused by members of Mucorales (genera are *Rhizopus*, *Rhizomucor*, *Mucor*, *Absidia*, *Aphphysomyces*, *Cunninghamella*, *Saksenaea*, etc.) should be named “mucormycosis” and are often associated with systemic spread (Waldorf 1989).

An extensive review by Roden et al. (2005) summarized the published reports of zygomycosis in the English literature since 1885 and found a total of 929 eligible cases. Summary statistics of that report indicate that the most common types of infection were sinus (39%), pulmonary (24%), cutaneous (19%), and systemic disease developed in 23% of the cases. The majority of the cases were males (65%). The reason for the higher prevalence rate in males is unknown. As

will be reviewed below, the majority of the cases were found to be associated with a few common risk factors associated with zygomycosis (i.e., diabetes, malignancies, etc.).

Iron is particularly important for the growth and virulence of Zygomycetes (Symeonidis, 2009). This means that physiological conditions resulting in increased bioavailability of iron predispose patients to Zygomycetes. Thus, most patients diagnosed with zygomycosis exhibit iron overload, as indicated by a high tissue iron burden, elevated serum transferrin, or increased non-transferrin-bound iron (Hogan et al., 1996; Ibrahim et al., 2012; Symeonidis 2009). There also appears to be a correlation with diabetic ketoacidosis and other acidoses, which predispose patients to zygomycosis by facilitating the dissociation of iron from iron-carrying proteins. Likewise, fungal infections caused by Zygomycetes are considered emerging infections in intensive care units (Paramythiotou et al., 2014) where patients are critically ill and susceptible to infections for a variety of reasons.

3.12.2.1 Site of Contact Effects

Rhinocerebral zygomycosis involves the nose and sinuses and is common in patients with poorly controlled diabetes mellitus as well as immunocompromised patients (Mantadakis and Samonis 2009). Symptoms are not unlike other causes of rhinosinusitis, and therefore, biopsy specimens help with histological diagnosis. *Conidiobolus coronatus* can infect the nasal mucosa (known as conidobolomycosis) after inhalation from the air or from soiled hands, as it is a common fungus found in the soil near decomposing plant materials (Yang et al., 2009). An unusual location of infection in an immunocompetent woman's vagina was reported after unsuccessful treatment for a yeast infection (Subramanian and Sobel, 2011). Consistent with other information on infections by Entomophthorales (the order into which the genus *Conidiobolus* falls), the infection did not spread.

Pulmonary zygomycosis is clinically and radiologically indistinguishable from pulmonary aspergillosis (Mantadakis and Samonis 2009). Patients usually present with prolonged fever and haemoptysis (coughing up blood or blood-stained mucus). The disease is common in neutropenic (low count of neutrophils, a type of white blood cell) patients and people with underlying hematological malignancies. A case of pulmonary infection after a stem cell transplant was reported by Matsumoto et al. (2014). A non-thrombotic (not a blood clot) pulmonary embolism (blockage of pulmonary artery) was caused by *Cunninghamella bertholletiae* (member of the Mucorales family).

Primary cutaneous zygomycosis is associated with traumatic inoculation of the skin in immunocompromised patients, burn victims and patients with other severe soft tissue trauma (Mantadakis and Samonis 2009). The initial clinical symptoms are indistinguishable from other cutaneous or subcutaneous infections, but necrotic eschars will appear if the infection is allowed to advance (Skiada and Petrikos, 2013). An outbreak of cutaneous mucormycosis in a pediatric

hospital was studied, and it was found that five cases (three fatal) were linked to hospital linens contaminated with *Rhizopus deleamar* (i.e., a species within the family Mucoraceae) (Duffy et al. 2014). A case of primary cutaneous mucormycosis caused by *Mucor irregularis* was also identified in the eye of a healthy 47-year old farmer who had recently undergone a dacryocystectomy (removal of lacrimal sac near ear). The patient recovered after treatment with antifungal medicine (Kang et al. 2014).

Gastrointestinal involvement is reported in the literature as “gastrointestinal zygomycosis” (Mantadakis and Samonis, 2009), “mucormycosis” (Mooney and Wagner, 1993), and “gastrointestinal basidiobolomycosis” (Yousef et al., 1999). Zygomycosis and mucormycosis cases were reported in infants who were suffering from prematurity, malnutrition, and immunosuppression. The basidiobolomycosis cases identified by these authors were from adults who were immunocompetent (as stated by the author), but later in the reference the patients had noted histories of peptic ulcer disease, alcohol abuse, diabetes mellitus or iron deficiency. Symptoms in the gastrointestinal cases included abdominal pain, weight loss, bloody discharge, anorexia, fever, anemia, and sometimes a palpable mass. Unfortunately, most cases are fatal and diagnosed at autopsy regardless of the age of the patient (Mooney and Wanger, 1993).

3.12.2.2 Irritation

No evidence was found that associated Zygomycetes with irritation-related health effects.

3.12.2.3 Sensitization, Inflammation, Allergy and Asthma

No evidence was found that associated Zygomycetes with health effects related to sensitization, inflammation, allergy or asthma.

3.12.2.4 Systemic Effects

Zygomycetes are capable of causing systemic effects due to the potential for organisms in the order Mucorales to invade blood vessels. Systemic zygomycosis usually stems from pulmonary zygomycosis and has been associated with severely immunocompromised patients (Mantadakis and Samonis, 2009). The risk factors of diabetes and iron overload are also associated with systemic effects. A report of a diabetic farmer experiencing systemic infection caused by *Saksenaea vasiformis* (a member of the order Mucorales) after introduction of the fungi via a head trauma highlights the importance of early detection and treatment (Gomez-Camarasa et al., 2014).

Secondary cutaneous (skin) zygomycosis is rare but possible. Secondary cutaneous zygomycosis is the result of the spread of an initiating infection (Mantadakis and Samonis, 2009). An unusual case without the observation of a primary focus of infection but with a diagnosis of cutaneous mucormycosis *after* fungemia (fungi in blood) was described by Dizbay et al. (2008) in an 83-year old diabetic woman. While in a neurological intensive care unit for left-sided weakness, the

patient exhibited worsening symptoms which led to the identification of *Mucor circinelloides* in her blood. Cutaneous lesions appeared 7 days after the fungemia was discovered and the CT scan of the lungs, paranasal sinuses and brain did not reveal a primary focus of infection.

3.12.3 Health Effects in Animals

The murine model has been used to test treatment efficacy for mucormycosis (Luo et al., 2014) and to investigate the increased virulence associated with the risk factor of iron overload (Ibrahim et al., 2012). Zygomycetes can also infect animals and cause health effects in sheep (Ubiali et al. 2013) and beef cattle (Nishimura et al. 2014) similar to those found in humans.

3.12.4 References

Dizbay, M., Adisen, E., Kustimur, S., Sari, N., Cengiz, B., Yalcin, B., Kalkanci, A., Gonul, II, Sugita, T., 2009. Fungemia and cutaneous zygomycosis due to *Mucor circinelloides* in an intensive care unit patient: case report and review of literature. *Jpn J Infect Dis.* 62, 146-148.

Duffy, J., Harris, J., Gade, L., Sehulster, L., Newhouse, E., O'Connell, H., Noble-Wang, J., Rao, C., Balajee, S. A., Chiller, T., 2014. Mucormycosis outbreak associated with hospital linens. *Pediatr Infect Dis J.* 33, 472-476. Abstract only.

Gómez-Camarasa, C., Rojo-Martín, M. D., Miranda-Casas, C., Alastruey-Izquierdo, A., Aliaga-Martínez, L., Labrador-Molina, J. M., Navarro-Marí, J. M., 2014. Disseminated infection due to *Saksenaea vasiformis* secondary to cutaneous mucormycosis. *Mycopathologia.* 177, 97-101. Abstract only.

Hogan, L. H., Klein, B. S., Levitz, S. M., 1996. Virulence factors of medically important fungi. *Clin Microbiol Rev.* 9, 469-488.

Ibrahim, A. S., Spellberg, B., Walsh, T. J., Kontoyiannis, D. P., 2012. Pathogenesis of mucormycosis. *Clin Infect Dis.* 54, 16-22.

Kang, D., Jiang, X., Wan, H., Ran, Y., Hao, D., Zhang, C., 2014. *Mucor irregularis* infection around the inner canthus cured by amphotericin B: a case report and review of published literatures. *Mycopathologia.* 178, 129-133.

Luo, G., Gebremariam, T., Lee, H., Edwards, J. E., Kovanda, L., Ibrahim, A. S., 2014. Isavuconazole therapy protects immunosuppressed mice from mucormycosis. *Antimicrob Agents Chemother.* 58, 2450–2453.

Mantadakis, E., Samonis, G., 2009. Clinical presentation of zygomycosis. *Clin Microbiol Infect.* 15 15-20.

Matsumoto, K., Yamamoto, W., Ohgusa, E., Tanaka, M., Maruta, A., Ishigatsubo, Y., Kanamori, H., 2014. Disseminated *Cunninghamella bertholletiae* infection with septic pulmonary embolism

after allogeneic bone marrow transplantation. *Transpl Infect Dis.* 16, 304-306. Abstract only.

Mooney, J. E., Wanger, A., 1993. Mucormycosis of the gastrointestinal tract in children: report of a case and review of the literature. *Pediatr Infect Dis J.* 12, 872-876.

Muszevska, A., Pawlowska, J., Krzyściak, P., 2014. Biology, systematics, and clinical manifestations of Zygomycota infections. *Eur J Clin Microbiol Infect Dis.* 33, 1273–1287.

Nishimura, M., Toyota, Y., Ishida, Y., Nakaya, H., Kameyama, K., Nishikawa, Y., Miyahara, K., Inokuma, H., Furuoka, H., 2014. Zygomycotic mediastinal lymphadenitis in beef cattle with ruminal tympany. *J Vet Med Sci.* 76, 123-127. Abstract only.

Paramythiotou, E., Frantzeskaki, F., Flevari, A., Armaganidis, A., Dimopoulos, G., 2014. Invasive fungal infections in the ICU: How to approach, how to treat. *Molecules.* 19, 1085-1119.

Ribes, J. A., Carolyn, L., Vanover-Sams, Baker, D. J., 2000. Zygomycetes in human disease. *Clin. Microbiol.* 13, 236-301.

Roden, M. M., Zaoutis, T. E., Buchanan, W. L., Knudsen, T. A., Sarkisova, T. A., Schaufele, R. L., Walsh, T. J., 2005. Epidemiology and outcome of zygomycosis: A review of 929 reported cases. *Clin Infect Dis.* 41, 634–653.

Skiada, A., Petrikkos, G., 2013 Cutaneous mucormycosis. *Skinmed.* 11, 155-159. Abstract only.

Subramanian, C., Sobel, J. D., 2011. A case of *Conidiobolus coronatus* in the vagina. *Med Mycol.* 49, 427-429.

Symeonidis, A. S., 2009. The role of iron and iron chelators in zygomycosis. *Clin Microbiol Infect.* 19, 26-32.

Ubiali, D. G., Cruz, R. A., De Paula, D. A., Silva, M. C., Mendonça, F. S., Dutra, V., Nakazato, L., Colodel, E. M., Pescador, C. A., 2013. Pathology of nasal infection caused by *Conidiobolus lamprauges* and *Pythium insidiosum* in sheep. *J Comp Pathol.* 149, 137-145. Abstract only.

Waldorf, A. R., 1989. Pulmonary defense mechanisms against opportunistic fungal pathogens. *Immunol Ser.* 47, 243-271.

Yang, X., Li, Y., Zhou, X., Wang, Y., Geng, S., Liu, H., Yang, Q., Lu, X., Hiruma, M., Sugita, T., Ikeda, S., Ogawa, H., 2009. Rhinofacial conidiobolomycosis caused by *Conidiobolus coronatus* in a Chinese rice farmer. *Mycoses.* 53, 369-373.

Yousef, O. M., Smilack, J. D., Kerr, D. M., Ramsey, R., Rosati, L., Colby, T. V., 1999. Gastrointestinal basidiobolomycosis. Morphologic findings in a cluster of six cases. *Am J Clin Pathol.* 112, 610-616.

4 Effects of Mycotoxins

Several of the genera produce more than one mycotoxin; therefore, this section is organized alphabetically by mycotoxins, rather than by genus. To facilitate review, Table 3 provides the genera, associated mycotoxins and the section where the major mycotoxins are discussed. Several of the genera produce numerous (tens to hundreds) of mycotoxins, but adequate information is available only for a few toxins. In these cases, general information on the classes of toxins is presented in the introduction to a group of toxins or in the context of the organism. Information on the mycotoxins produced by the various genera is based on the best available information. However, it was often difficult to determine definitively which mycotoxins are produced by which genus. In some cases this reflects differences in toxin production by different strains or species within a genus. In other cases, this reflects data gaps, or the tendency of review articles to focus on primary toxins and classes of toxins, rather than identifying each toxin (or each major toxin) produced by a genus. Table 3 summarizes the toxins produced by each genus/class, and the section that the toxin(s) is addressed.

Exposure to mold spores may occur via all three routes (dermal contact with the mold, oral exposure via hand to mouth contact or contamination of food, or inhalation exposure to airborne spores). Some molds (particularly *Stachybotrys*) produce volatile compounds, but it is not well understood how other mycotoxins may become airborne (Gareis and Gottschalk, 2014). One mechanism may be the formation of droplets from droplets exuded by the mold colony, a process called guttation. For example, guttation droplets containing mycotoxins have been reported for colonies of *Stachybotrys* (containing macrocyclic trichothecenes such as satratoxins G and H) (Gareis and Gottschalk, 2014) and *Penicillium* (containing ochratoxins A and B) (Gareis and Gareis, 2007)³.

Table 3. Summary of Toxins Associated with Organisms Addressed in this Document

Genus/Class	Toxin Production	Section of Report
Alternaria	Alternariol and related Alttoxins Tetramic acids	Section 4.2 Section 4.2 Section 4.2

³ Gareis, M., Gottschalk, C. 2014. *Stachybotrys* spp. and the guttation phenomenon. *Mycotoxin Res.* 20:151-9. doi: 10.1007/s12550-014-0193-3.

Gareis, M, Gareis, E.M. 2007. Guttation droplets of *Penicillium nordicum* and *Penicillium verrucosum* contain high concentrations of the mycotoxins ochratoxin A and B. *Mycopathologia.* 163::207-14.

Genus/Class	Toxin Production	Section of Report
Aspergillus	Aflatoxin Citrinin Ochratoxin Sterigmatocystin Various other toxins	Section 4.1 Section 4.5 Section 4.6 Section 4.7 Section 3.3
Chaetomium	Chetoglobosins Chaetochromin Cytochalasins Various other toxins	Section 4.3 Section 4.3 Section 4.3 n/a ¹
Cladosporium	None	n/a
Dicyma	Various minor toxins	n/a
Epicoccum	None	n/a
Malassezia	None	n/a
Penicillium	Citrinin Ochratoxin	Section 4.5 Section 4.6
Phoma	Various minor toxins	n/a
Stachybotrys	Cyclosporine Trichothecenes, including satratoxins Various other toxins	Section 4.4 Section 4.8 n/a
Rhizopus (Zygomycetes)	No	n/a

¹n/a = not applicable

4.1 Aflatoxin

4.1.1 Physical and Chemical Characteristics

Table 4. Physical and Chemical Characteristics of Aflatoxins

Characteristic		Reference
Molecular formula	Aflatoxin B1 (AfB1): C ₁₇ H ₁₂ O ₆ Aflatoxin B2 (AfB2): C ₁₇ H ₁₄ O ₆ Aflatoxin G1 (AfG1): C ₁₇ H ₁₂ O ₇	IARC, 1993

Characteristic		Reference
	Aflatoxin G2 (AfG2): C ₁₇ H ₁₄ O ₇ Aflatoxin M1 (AfM1): C ₁₇ H ₁₂ O ₇	
IUPAC	AfB1: Name: (6aR- <i>cis</i>)(2,3,6a,9a)Tetrahydro-4-methoxycyclopenta[<i>c</i>]furo[3',2':4,5]furo[2,3- <i>h</i>][<i>l</i>]benzopyran-1,11-dione AfB2: (6aR- <i>cis</i>)(2,3,6a,8,9,9a)Hexahydro-4-methoxycyclopenta[<i>c</i>]furo-[3',2':4,5]furo[2,3- <i>h</i>][<i>l</i>]benzopyran-1,11-dione AfG1: (7aR- <i>cis</i>)(3,4,7a,10a)Tetrahydro-5-methoxy-1 <i>H</i> ,12 <i>H</i> -furo[3',2':4,5]furo(2,3- <i>h</i>)pyrano[3,4- <i>c</i>][<i>l</i>]benzopyran-1, 12-dione AfG2: (7aR- <i>cis</i>)(3,4,7a,9,10,10a)Hexahydro-5-methoxy-1 <i>H</i> ,12 <i>H</i> -furo-[3',2':4,5]furo[2,3- <i>h</i>]pyrano[3, 4- <i>c</i>][<i>l</i>]benzopyran-1, 12-dione AfM1: 2,3,6a, 9a-Tetrahydro-9a-hydroxy-4-methoxycyclopenta[<i>c</i>]furo-[3',2':4,5]furo[2,3- <i>h</i>][<i>l</i>]benzopyran-1, 11-dione	IARC, 1993
CAS No.	AfB1: 1162-65-8 AfB2: 7220-81-7 AfG1: 1165-39-5 AfG2: 7241-98-7 AfM1: 6795-23-9	IARC, 1993
Molecular weight	AfB1: 312.3 g/mol AfB2: 314.3 g/mol AfG1: 328.3 g/mol AfG2: 330.3 g/mol AfM1: 328.3 g/mol	IARC, 1993
Melting Point and Decomposition	AfB1: 262-269 °C (decomposition) (crystals from chloroform) AfB2: 287-289 °C (decomposition) (crystals from chloroform-pentane) AfG1: 244-246 (decomposition) (crystals from chloroform-methanol) AfG2: 237-239 (decomposition) (crystals from ethyl acetate) AfM2: 299 (decomposition) (crystals from methanol)	IARC, 1993
Physical State	Colourless to pale-yellow crystals. Intensely fluorescent in ultraviolet light, emitting blue (AfB1 and AfB2) or yellow-green (AfG1, AfG2) fluorescence, from which the designations AfB and AfG were derived, or blue-violet fluorescence (AfM1)	IARC, 1993
Solubility	Very slightly soluble in water (10-30 µg/ml); insoluble in non-polar solvents; freely soluble in moderately polar organic solvents (e.g., chloroform and methanol) and especially in dimethyl sulfoxide.	IARC, 1993
Stability	Unstable to ultraviolet light in the presence of oxygen, to extremes of pH (<3, >10) and to oxidizing agents.	IARC, 1993

Aflatoxins are toxic secondary metabolites produced by three species of *Aspergillus* – *A. flavus*, *A. parasiticus*, and the rare *A. nomius*. *A. flavus* produces only B aflatoxins, while the other two species produce both B and G aflatoxins. Aflatoxins M1 and M2 are the hydroxylated metabolites of aflatoxins B1 and B2 (IARC, 2012; JECFA, 2001; Peraica et al., 1999).

Aflatoxicol is a reductive metabolite of aflatoxin B1. Aflatoxins are structurally closely related to the polyketide mycotoxin, sterigmatocystin, produced by several fungal species and which shares its biosynthetic pathway with aflatoxins (EFSA, 2013).

4.1.2 Toxicokinetics

Several studies evaluating the toxicokinetics of aflatoxins are available via the oral, dermal, inhalation, intravenous (iv), intraperitoneal (ip), and intratracheal instillation routes; *in vitro* studies have also evaluated aflatoxin metabolism (IARC 1993, 2002). Although the absorption of aflatoxins has not been quantified, animal studies indicate that aflatoxins are readily absorbed following oral, inhalation and dermal exposure (IARC, 2012). After absorption, aflatoxins are metabolized in the liver to an epoxide that binds macromolecules, especially nucleic acids and nucleoproteins (IARC, 2012). Absorption after intratracheal absorption is faster than following oral exposure, but a similar distribution and excretion pattern is seen for both routes (IARC, 2012).

Aflatoxins B1 and M1 are concentrated in the liver of rats 30 minutes after an oral or ip dose of 7 mg/kg ¹⁴C-AfB1; at 24 hours, both aflatoxins were detected only in trace amounts (IARC, 2012). Aflatoxins have been detected in the blood of pregnant women, in neonatal umbilical cord blood, and in breast milk in African countries, with significant seasonal variations (Peraica et al., 1999). Aflatoxins can cross the placenta (IARC, 1993), consistent with the observed developmental effects (e.g., fetal anomalies and implantation loss – see Section 4.1.4.4).

The metabolism of AfB1 in humans and laboratory animals has been well characterized (IARC, 2012; JECFA, 2001). Aflatoxins undergo phase I and phase II metabolism, mediated by cytochrome P450 (CYP) enzymes and glutathione S-transferase M1 (GST M1), respectively. CYP enzymes metabolize AfB1 to generate two chemically reactive epoxides in humans and other species: AfB1-8,9-*exo* and -8,9-*endo* epoxides. However, only AfB1 8,9-*exo*-epoxide reacts readily with DNA to form the N7-guanine and its derivative AfB1-formamidopyrimidine adducts (IARC, 2012; Johnson and Guengerich, 1997). CYP enzymes also generate the hydroxyl derivatives of AfB1. The highly unstable, highly reactive 8,9-*exo* isomer binds to biological nucleophiles (e.g., nucleic acids) to form stable links to RNA and DNA, induce point mutations and DNA strand breaks, and thus, it is the main mediator of cellular injury (IARC, 2012). Aflatoxins also form aflatoxin–albumin adducts (Gupta, 2011).

There are marked species and/or strain differences in sensitivity to aflatoxin-induced carcinogenesis; these are due to the differences in activation and detoxification activities of the

aflatoxin-metabolizing enzymes. Adult mice are almost completely refractory to tumor formation, while the rat is extremely sensitive. This difference is explained by the fact that, compared to rats, mice have higher levels of constitutive GST isoforms with high AfB1 8,9-epoxide-conjugating activity, thus deactivating the active metabolite (IARC, 2012). The amount of AfB1 bound to macromolecules was much lower in human liver slices than in rat liver slices, indicating that, compared to rats, humans do not form as much AfB1 8,9-epoxide. However, the data also indicate that humans do not have GST isozymes with high specific activity towards this epoxide, making humans more like rats than mice in this aspect (JECFA, 2001). Because of the importance of GST M1 isozyme in detoxifying the reactive epoxide, polymorphisms in the *GST-M1* gene may be important in identifying susceptible populations. Data suggest that people with the *GST-M1*-null genotype are at higher risk for liver cancer (IARC, 2012).

Excretion of AfB1 metabolites occurs primarily through the biliary pathway, followed in importance by the urinary pathway. AfM1 is the major unconjugated AfB1 metabolite in the rat, monkey, and humans, while AfQ1 is the major metabolite in the mouse (Eaton and Groopman, 1994). Elimination was relatively slow in all species and strains studied. Following iv dosing, mice, which are less susceptible, produced the most water-soluble urinary metabolites, while monkeys and rats, which are more susceptible, produced less of these metabolites in the urine. Similar results were reported in studies with different species and different routes of administration. In rats, the major biliary AfB1 metabolites after oral radiolabeled AfB1 administration were AfB1-glutathione (AfB1-GSH) conjugates. Following ip injection of AfB1 in rats, the three major urinary metabolites were AfM1, AfP1, and AfB1-N7-guanine, with AfM1 being the major recovered metabolite. In humans, the concentration of fecal AfQ1 was approximately 60 times higher than that of AfM1 (Mykkanen et al., 2005). In human urine, the median AfQ1 concentration was 27 and 260 times higher than that of AfB1-N7-guanine (the major AfB1-DNA adduct) and AfM1, respectively (Mykkanen et al., 2005). In the same study in humans, AfQ1 was excreted in urine and feces at higher levels than AfM1, and feces were an important route of excretion of these AfB1 metabolites. In monkeys after iv dosing, AfM1 was also the major AfB1 metabolite. Unlike the situation in the rat, monkey and human, in the mouse, AfP1, rather than AfM1, is the major AfB1 metabolite (Eaton and Groopman, 1994). The mouse is the only species that has been found to excrete AfQ1 as a urinary metabolite (Eaton and Groopman, 1994). More AfB1 metabolites (and conjugated compounds) are usually excreted in rat feces than in urine after ip injection of [¹⁴C]-ring-labelled aflatoxin B1 (IARC, 2012).

4.1.3 Hazard Information - Human

The adverse effects of aflatoxins in humans range from acute hepatic toxicity to chronic disease, such as liver cancer (Agag, 2004; Peraica et al., 1999). Human data suggest that children are more vulnerable than adults to acute hepatotoxicity resulting from ingestion of aflatoxin (IARC, 2002).

In tropical countries where several outbreaks of aflatoxicosis [the poisoning that results from ingesting aflatoxins, of which severe symptoms include hemorrhagic necrosis of the liver, bile duct proliferation, edema, and lethargy (Williams et al., 2004)] have occurred in adults in rural populations with poor level of nutrition, the clinical picture indicates acute toxic liver injury (toxic hepatitis) (Peraica et al., 1999). Mortality rates in the acute phase were 10-60 %. Although no information is available on the doses ingested, the doses are likely to be high, in light of the high mortality rate. A young woman ingesting a total of 5.5 mg of AfB1 over 2 days presented with a transient, nonpruritic, macular rash, nausea and headache. When the same woman, 6 months later, ingested a total of 35 mg over 2 weeks, she reported only nausea. There was no sign of hepatotoxicity, suggesting that hepatotoxicity of AfB1 may be lower in well nourished persons than in experimental animals (Peraica et al., 1999).

Aflatoxins have been suggested as an etiological factor in encephalopathy and fatty degeneration of viscera, similar to Reye syndrome (Peraica et al., 1999). The clinical picture includes enlarged, pale, fatty liver and kidneys and severe cerebral edema, but use of aspirin or phenothiazines is also suspected to be involved in the etiology (Peraica et al., 1999).

Limited information is available about the effects of inhaled aflatoxins. Inhalation exposure in an industrial or farm setting has been associated with cancers of the liver, intestine, and kidney in animals and humans, as well as the lung in humans (EPA, 2004).

As in animals, adverse effects of aflatoxins on the embryo and the developing fetus (such as regression of testis, impairment of spermatogenesis and premature loss of germ cells) have been reported in humans (Gupta, 2011). These findings were correlated with the presence of higher concentrations of aflatoxins in the semen of infertile men (40% of cases compared to 8% of controls). Aflatoxins were also reported to lower fertility and significantly increased mortality of embryos in humans (Gupta, 2011), but information on effect levels in humans was not located.

Several epidemiological studies (observational studies, correlation studies, and case-control and cohort studies) with AfB1 provide evidence that dietary intake of aflatoxins is a cause of liver cancer (extensively reviewed by IARC, 1993, 2002, 2012; JECFA, 2001). Although the levels of exposures to the aflatoxins have not been determined or reported, cohort studies show a positive correlation between populations exposed to AfB1 and hepatocellular carcinoma (HCC) (IARC, 2012). Although the increased risk for HCC was reported to be independent of exposure to hepatitis B virus (HBV), case-series and case-control studies also confirmed that the carcinogenic potency of AfB1 is substantially higher in carriers of HBV who are exposed to aflatoxin in their diets (JECFA, 2001; IARC, 1993, 2002, 2012).

Aflatoxins also have the potential to induce a specific mutation in codon 249 of the *TP53* tumor-suppressor gene (IARC, 2012), mutations of which are the most common alteration in cancer. This observation further supports the role of aflatoxins in the development of HCC.

4.1.4 Hazard Information - Animal

Aflatoxins cause liver damage, decreased milk production, reproductive toxicity and suppressed immunity in animals consuming low dietary concentrations (Agag, 2004). Rats are more sensitive than mice, and Fischer rats the most sensitive strain (Gupta, 2011). Rabbits are also highly susceptible to acute doses. Following acute exposure, the clinical signs include gastrointestinal dysfunctions, decreased feed intake and efficiency, weight loss, jaundice, drop in milk production, nervous signs, bleeding and death (Agag, 2004). Most species of animals are susceptible to aflatoxicosis (Williams et al., 2004). The susceptibility of individual animals to aflatoxicosis varies considerably depending on dose, duration of exposure, species, age, sex, and nutrition (Agag, 2004). “Across all species, the dose and duration of exposure to aflatoxin clearly have a major effect on the toxicology and may cause a range of consequences: 1) large doses lead to acute illness and death, usually through liver cirrhosis; 2) chronic sublethal doses have nutritional and immunologic consequences; and 3) all doses have a cumulative effect on the risk of cancer” (Williams et al., 2004).

4.1.4.1 Acute Lethality

There is a wide variation in LD₅₀ values in animal species tested with single doses of aflatoxins. AfB1 is the most potent of the aflatoxins. The oral LD₅₀ values range from 0.3 mg/kg in rabbits to 17.9 mg/kg in rats (Guevara-González, 2011; McKean et al., 2006; Agag, 2004; IARC, 1993). The order of potency of the different aflatoxins in both acute and chronic studies is AfB1>AfG1>AfB2>AfG2 (IARC, 2012).

4.1.4.2 Acute/Short-term Toxicity

The limited data available on the skin irritative potential of aflatoxins indicate that they act as primary skin irritants in rabbits (Joffe and Ungar, 1969). However, no data were identified regarding the sensitization potential.

In each species tested, the liver is the primary target organ of acute effects of aflatoxins (FDA, 2013). In the rat, the main lesions following the administration of AfB1 at high doses were seen in the liver, while kidney and adrenals also show damage (Talebi et al., 2011). AfM1 has a very similar acute toxicity to that of AfB1 (Butler, 1964). Most of the acute/short-term toxicity studies in the rat and dog reported on mortality following oral exposure to AfB1 (IARC, 1993, McKean et al., 2006; Newberne et al., 1966). While a single oral dose of AfB1 of 0.5 mg/kg did not result in any mortality in the dog (Newberne et al., 1966), the lowest lethal oral dose in the rat was 2.15 mg/kg (McKean et al., 2006); no deaths were seen in rats at 1 mg/kg in this study.

In rats, AfB1 given via oral gavage at a dose of 0.04 mg per day (the only dose tested) for 10 days (total dose 0.4 mg/rat) (approximately equal to 0.05 mg/kg-day, based on initial weights of the rats) resulted in no mortality attributable to acute toxicity (IARC, 1993). However, deaths occurred in rats given approximately 1 mg AfB1/kg by oral gavage for five days (IARC, 1993).

In the dog, a single oral dose of 0.5 mg/kg AfB1 resulted in liver lesions that were clearly apparent within 3 days, with only minimal regression up to 14 days later (Newberne et al., 1966). Repeated oral dosing of dogs with 0.2 mg/day AfB1 for 15 days did not result in mortalities up to 13 days after dosing was discontinued, but did result in moderate to severe liver lesions.

4.1.4.3 Repeated Dose Toxicity

Aflatoxins have been found to be moderately to highly toxic in almost every animal species tested, including monkeys, although they do not affect all animals equally (FDA, 2013). The liver is also the main target organ following repeated dose toxicity.

Although no standard repeated dose toxicity studies were identified, data were identified in experimental animals exposed to diets containing defined amounts of AfB1, or contaminated naturally with aflatoxins, including diets treated with ammonia to render them suitable for feed (IARC, 1993, 2002). These studies reported mainly on mortality, and a limited number reported on systemic effects following exposure. Deaths occurred when male Fischer 344 rats were fed a diet containing a mixture of AfB1 (1 ppm and 0.17 ppm AfG1; equivalent to about 0.08 mg/kg-day AfB1 and 0.01 mg/kg-day AfG1), but no deaths were reported when the animals were fed an ammoniated diet containing 0.06 ppm (0.005 mg/kg-day) AfB1 and 0.01 ppm (0.0008 mg/kg-day) AfG1 or a diet containing 0.05 ppm AfB1 (0.004 mg/kg-day) for 12 months (IARC, 1993). However, when Sprague Dawley rats were fed a diet containing aflatoxins (AfB1 was present at a level of 0.005-0.0075 ppm) (0.0004-0.0006 mg/kg-day) for 22 months, the animals showed parenchymal liver damage (swelling of cytoplasm, fatty infiltration) (IARC, 1993). Although studies are limited, it appears that the oral LOAEL for systemic effects is 0.0004 mg/kg-day (the lowest dose tested from the 22-month study).

4.1.4.4 Developmental and Reproductive Toxicity

The reproductive and developmental effects of aflatoxins have been reviewed (IARC, 1993; Gupta, 2011). The available studies indicate that AfB1 impairs the reproductive performance of females, resulting in adverse effects on sexual maturation, growth and maturation of the follicles, levels of hormones, gestation and growth of the fetus (Gupta, 2011). Male reproductive toxicity studies with aflatoxins *in vivo* and *in vitro* have reported testicular degeneration and decreased sperm production (Gupta, 2011).

While no standard oral or dermal reproductive or developmental toxicity studies were identified for aflatoxins, in female rats orally administered AfB1 at doses of 7.5 or 15 mg/kg-day for 21 days, significant, dose-dependent reductions in the number of oocytes and large follicles were observed, together with significant alteration of blood hormone levels and sex organ weights (Ibeh and Saxena, 1997b; Gupta, 2011). In a related study, decreased conception rate and litter size, and increases in fetal resorption and implantation loss were seen in rats gavaged with AfB1 at 7.5 mg/kg-day for 14 days (Ibeh and Saxena, 1997a; Gupta, 2011).

In mice, oral administration of 4 mg/kg AfB1 on day 8 or 9 of pregnancy resulted in fetal anomalies including exencephaly (brain is located outside of the skull), open eyes and protrusion of intestines in fetuses exposed on day 8. No teratogenicity was seen in fetuses exposed on day 9 (IARC, 1993), but the number of treated litters was relatively low. Alternatively, the observations on day 8 may reflect factors other than AfB1 exposure (e.g., study quality); this study was not cited in the 2002 IARC report. Orally treating a group of mice at a dose level of 45 mg/kg of AfB1 on gestation days 12 and 13 produced no developmental or teratogenic effects (IARC, 1993), perhaps because the fetuses were past the window of susceptibility. Malformations have also been observed following parenteral dosing at 32 mg/kg and higher.

Male Swiss albino mice fed a diet containing aflatoxins at a concentration of 0.078 ppm (equivalent to 0.01 mg/kg-day, based on male mouse average food factor of 0.191 kg food/kg-day) for 7, 14, 21, 28 or 35 days developed duration-dependent statistically significant increase in abnormal sperm cells compared with the negative controls (Ezekiel et al., 2011). Administration of 0.015 or 0.03 mg/kg AfB1 to adult male rabbits every other day for 9 weeks (average doses of 0.0075 or 0.015 mg/kg-day) resulted in decreased body weight, relative testis weight, serum testosterone, sperm concentration and motility (IARC, 2002). An ip administration of AfB1 to male Swiss albino mice at a dose of 0.05 mg/kg-day (the only dose tested) for 7, 15, 35 or 45 days also resulted in a decreased sperm concentration and motility and an increase in abnormalities (Agnes and Akbarsha, 2003).

Based on the limited data available, a single dose of 4 mg/kg AfB1 may cause developmental toxicity, although, as noted, this study is of poor quality. Male reproductive toxicity was reported following dosing with 0.01 mg/kg-day for up to 35 days, or 0.015 mg/kg-day every other day for 9 weeks. Effects on female reproduction were seen at 7.5 mg/kg-day for 14 days. No clear NOAEL has been identified for developmental or reproductive effects of AfB1.

4.1.4.5 Immunotoxicity

The immunotoxic potential of AfB1 has been investigated *in vivo* and *in vitro*, and available data indicate that aflatoxins compromise immunity in farm and laboratory animals exposed chronically to aflatoxins (reviewed in Williams et al., 2004). However, no threshold for

immunotoxicity has been defined for any species (Williams et al., 2004), and most of the information in key reviews does not include exposure durations. The primary immunosuppressive effect of aflatoxins is on cell-mediated immunity, particularly delayed-type hypersensitivity. Reduced antibody secretion and altered cytokine levels have also been reported (IARC, 2002). In rats and mice as well as other animals, AfB1 induced thymic aplasia (i.e., the failure of the thymus to develop or to function normally), reduced T-lymphocyte function and number, suppressed phagocytic activity, and reduced complement activity (Williams et al., 2004).

A dose of 0.75 mg/kg of AfB1 in mice (route not specified) significantly decreased splenic CD4 (helper T) cell numbers and interleukin 2 (IL-2) production. An oral dose of 0.03-0.07 mg/kg AfB1 given to BALB/c mice for an unspecified duration suppressed natural killer (NK) cell-mediated cytotoxicity of YAC-1 target cells. No such effects were observed in C57B1/6 mice given the same dose of AfB1 or in rabbits fed 24 ppm aflatoxin in feed. When male Wistar rats were fed a diet containing 0.04 ppm (equivalent to 0.004 mg/kg-day) AfB1 for 90 days, the mitotic response of spleen mononuclear cells *in vivo* was higher compared to controls. Aflatoxins were also reported to reduce antibody titers to some infectious bacteria in rabbits (Williams et al., 2004). Aflatoxin has also been shown to reduce phagocytic activity in rabbit alveolar macrophages and to inhibit phagocytic cell function in normal peripheral blood monocytes *in vitro* (Williams et al., 2004).

4.1.4.6 Genotoxicity

Numerous studies have been conducted on the genotoxicity of aflatoxins, and they have been characterized as potent mutagens (Gupta, 2011). The genotoxic potential of AfB1 has been demonstrated in prokaryotic and eukaryotic systems *in vitro*, including human cells, as well as *in vivo* in humans and several animal species (IARC, 2002). AfB1 forms adducts with DNA and is positive in bacterial and mammalian gene mutation assays (IARC, 2002). AfB1 is also reported to cause mutation in germ cells (Ezekiel et al., 2011). The mutagenic effects are much stronger following metabolic activation. AfB1 is also clastogenic, resulting in the formation of micronuclei and chromosome aberrations in animals and human cells (IARC, 2002). AfB1 is also positive in a variety of tests for DNA damage, including unscheduled DNA synthesis (UDS), sister chromatid exchange (SCE), and mitotic recombination (IARC, 2002; Smerak et al., 2001; Ezekiel et al., 2011). AfM1 was also reported to be a DNA-damaging agent, with an activity about one-third that of AfB1 (JECFA, 2001).

4.1.4.7 Carcinogenicity

Although no standard chronic toxicity/carcinogenicity studies were available for the aflatoxins, there is an extensive database of nonstandard studies that investigated aflatoxin carcinogenicity. These studies include single dose, acute/short-term, repeated dose, and chronic exposure studies

in which the experimental animals were fed diet naturally or artificially contaminated with AfB1, mixtures of aflatoxins or AfM1. Irrespective of the exposure duration, studies in rats have demonstrated the carcinogenic potency of aflatoxins (IARC, 1993, 2002, 2012). Results from these studies indicate that AfB1 is a very potent carcinogen in many species, including nonhuman primates and rodents.

The main target organ for carcinogenicity is the liver, causing hepatocellular carcinomas in rats. AfB1 also causes a variety of other tumors, including colon and kidney tumors (rats); lung adenomas (mice); cholangiocellular tumors (hamsters); and osteogenic sarcoma, adenocarcinomas of the gall bladder and carcinoma of the pancreas (monkeys) (Gupta, 2011; IARC, 2002, 2012). However, oral administration of AfB1 failed to induce liver tumors in mice (IARC, 2012), indicating a species difference in the carcinogenic response to aflatoxins (see additional information below). Cholangiosarcomas and liver cell carcinomas were seen in rats exposed to AfB1 *in utero*, with or without postnatal treatment; the dams were exposed via the diet (IARC, 1993). JECFA (2001) estimated the potency of AfM1 to be 10% of that of AfB1, based on studies in rats, while others concluded that the carcinogenicity of AfM1 is probably one to two orders of magnitude lower than that of AfB1 (IARC, 2012).

A variety of preneoplastic liver lesions have been reported following single dose and/or acute/short-term, and repeated dose studies with AfB1, including nodular hepatic hyperplasia, cystic bile-duct hyperplasia, foci of altered hepatocytes, foci of hyperplasia and transitional cells and hyperplastic nodules (IARC, 1993).

4.1.4.8 *In Vitro Studies and Data Related to Mode of Action*

Several *in vitro* and *in vivo* studies have been conducted to investigate the mode of action of the aflatoxins for mutagenic/carcinogenic effects as well as for other potential forms of toxicity (IARC, 1993, 2002, 2012; Agag, 2004; Williams et al., 2003). The mutagenicity and carcinogenicity of the aflatoxins are considered to arise as the result of the formation of a reactive 8,9-*exo*-epoxide and its subsequent covalent binding to nucleic acid to form the promutagenic AfB1-*N7*-guanine DNA adduct that results in G→T transversion mutations in codon 249 of the *TP53* tumor-suppressor gene (IARC, 2012; Agag, 2004). The epoxide also induces gene mutations and chromosomal alterations (including micronuclei and sister chromatid exchange) and mitotic recombination (IARC, 2012). IARC (2012) noted that the key steps in the carcinogenic mode of action of aflatoxins involve the following: a) metabolism to the reactive *exo*-epoxide, b) binding of the epoxide to DNA, resulting in formation of DNA adducts; and c) miscoding in replicating DNA. This leads to the development of mutations that eventually progress to tumors. The same metabolic pathways that convert AfB1 to the 8,9-epoxide, forming DNA and albumin adducts in susceptible animals (rats) are active in humans, with the levels of these adducts in humans being comparable to those in susceptible species (IARC, 2012). The

damage to DNA is reduced following conjugation of glutathione to the 8,9-epoxide, a reaction mediated by GST. This conjugation is important in reducing the tumor burden in experimental animals. Species, such as the mouse that have elevated constitutive GST activity, are resistant to aflatoxin carcinogenesis (IARC, 1993). Humans do not form as much aflatoxin B₁ 8,9-epoxide as rats, but humans are more like rats than mice in their levels of GST isozymes (IARC, 2012).

IARC (2002) noted that biological interactions with HBV also play a role in the hepatic carcinogenicity of aflatoxins in humans. It is also suggested that the formation of reactive oxygen species and lipid peroxidation also play a major role in aflatoxin toxicity (Ezekiel et al., 2011).

Table 5. Summary of Aflatoxin Toxicity Data

Study Type	Route, Duration	References
Toxicokinetics	Several oral, dermal, iv, ip, intratreacheal instillation, and in vitro studies	IARC, 1993, 2002, 2012
Acute lethality	Oral LD ₅₀ s range from 0.3 mg/kg in the rabbit to 17.9 mg/kg in the rat	McKean et al., 2006; Agag, 2004; IARC, 1993
Acute/short-term toxicity	Limited standard toxicity studies. 1 single oral study each in rats and dogs; 1 oral study each in rats for 5, 10, and 15 days; 1 study (route not specified) and 1 oral/feeding study each in mice and rabbits for immunotoxicity; additional parenteral studies	Williams et al., 2004; IARC, 1993
Primary Irritation	1 skin irritation study in rabbits	Joffe and Ungar, 1969
Sensitization	No data identified	
Repeated dose toxicity	1 dietary study each in rats for 12 and 22 months; 1 dietary study in in rats for immunotoxicity for 90 days	IARC, 1993, 2002
Developmental	No standard studies. 1 oral study in rats each for 14 and 21 days; 4 oral studies in mice (exposures on GD 8 or 9, GDs 12 and 13, and for 7-35 days); 1 ip study in mice for 7-45 days	Gupta, 2011; IARC, 1993; Ezekiel et al., 2011; Agnes and Akbarsha, 2003
Reproductive	No standard studies. 1 oral (35 days) and 1 ip study (up to 45 days) of male reproductive toxicity in mice	Ezekiel et al., 2011; Agnes and Akbarsha, 2003
Genotoxicity	Numerous <i>in vivo</i> and <i>in vitro</i> tests for gene mutation, chromosome damage, and DNA damage	Gupta, 2011; IARC, 2002; Smerak et al., 2001; Ezekiel et al., 2011; JECFA, 2001
Carcinogenicity	Numerous single dose, acute/short-term, repeated dose, and chronic exposure studies	IARC, 1993, 2002, 2012; Gupta, 2011; JECFA 2001

4.1.4.9 **Lowest Hazard Endpoints**

The liver is the primary target organ of acute injury from aflatoxin exposure, although the kidney and adrenals also show damage. AFB1 is the most potent aflatoxin in both humans and experimental animals. Although the effects in humans are consistent with those seen in experimental animals, data on effect levels in humans is limited.

Most of the acute/short-term and repeated dose toxicity studies identified reported on mortality, with only a few reporting on systemic effects. Mortality was seen at a single dose as low as 2.15 mg/kg in the rat (McKean et al., 2006). The available data were insufficient to identify a short-term NOAEL for liver effects. The liver is also the main target organ following repeated dose toxicity. Although no standard repeated dose toxicity studies are available, the oral LOAEL appears to be 0.0004 mg/kg-day (there was no NOAEL) from a 22-month study in rats, based on parenchymal liver damage (swelling of cytoplasm, fatty infiltration) (IARC, 1993). The NOAEL/LOAEL for mortality is 0.004/0.08 mg/kg-day AFB1 (IARC, 1993), based on a 12-month study.

Aflatoxins are primary skin irritants (Joffe and Ungar, 1969), but data were not available on the potential of aflatoxins to cause skin sensitization.

There are no standard reproductive or developmental toxicity studies for the aflatoxins. However, single high and repeated dose studies show the potential of AFB1 to impair reproductive performance of female animals (IARC, 1993; Gupta, 2011), with effects seen in rats gavaged with 7.5 mg/kg-day for 14 days (Ibeh and Saxena, 1997a). *In vivo* and/or *in vitro* studies identified the testes as a sensitive target for aflatoxins, with effects on various aspects of spermatogenesis (Gupta, 2011; Ezekiel et al., 2011). Male reproductive toxicity was reported following dosing with 0.01 mg/kg-day for up to 35 days (Ezekiel et al., 2011), or 0.015 mg/kg every other day for 9 weeks (IARC, 2002). A single dose of 4 mg/kg AFB1 during a susceptible window may cause developmental toxicity, including malformations (IARC 1993). Although malformations were also seen at high parenteral doses, the reliability of the report is low. No NOAEL was identified for any reproductive or developmental endpoint.

Aflatoxins have the potential to be immunotoxic. The primary effect is on cell-mediated responses, but antibody production can also be affected (IARC, 2002). Dose-response data are limited, but effects were seen in mice at an oral dose of 0.03 mg/kg for an unspecified duration (Williams et al., 2004); no NOAEL was identified.

The aflatoxins form DNA adducts, resulting in gene mutations in a wide range of *in vitro* and *in vivo* studies (IARC, 1993, 2002, 2012). No standard carcinogenicity studies were identified. However, a series of studies including single dose, acute/short-term, repeated dose, and chronic exposure studies have evaluated the carcinogenic potential of aflatoxins, and found that

aflatoxins were clearly positive. The International Agency for Research on Cancer (IARC) has assessed the carcinogenic potential of aflatoxins (IARC, 1993, 2002, 2012). IARC has concluded that aflatoxins are *carcinogenic to humans (Group 1)* (IARC, 1993, 2002, 2012) based on sufficient evidence for carcinogenicity in humans, causing hepatocellular carcinoma, and sufficient evidence in experimental animals for the carcinogenicity of naturally-occurring aflatoxins, as well as AfB1, AfG1, and AfM1. Based on studies in rats, JECFA (2001) estimated the potency of AfM1 to be 10% of that of AfB1. AfB1 is a potent liver carcinogen in a number of animal species, although wide species variability exists. It causes liver tumors in mice, rats, fish, marmosets and monkeys following administration by various routes.

Overall, the adverse effects of aflatoxins in humans ranged from acute hepatic toxicity to chronic disease, such as liver cancer (Agag, 2004; Peraica et al., 1999; IARC, 1993, 2002, 2012). Data in humans also suggest that children are more vulnerable than adults to acute hepatotoxicity resulting from ingestion of aflatoxin (IARC, 2002). Species and/or strain differences to aflatoxin-induced carcinogenesis have been noted. These differences have been attributable to the differences in activation and detoxification activities of the aflatoxin-metabolizing enzymes. Studies suggest that people with the *GSTM1* genotype are at increased risk for liver cancer from aflatoxins, but the data currently available were based on relatively small cohorts in the high-risk strata (IARC, 2012). People with HBV infection are also more susceptible to the development of hepatocellular carcinoma from aflatoxin exposure (IARC, 2012).

Table 6. Lowest Hazard Endpoints for Aflatoxin

Species/ Route	Duration	NOAEL/ LOAEL	Effect/Comments	Citation
	Acute and short-term		No good-quality NOAELs or LOAELs identified	
Rat/oral	22 months	No NOAEL; LOAEL 0.0004 mg/kg-day	Parenchymal liver damage, high confidence on endpoint	IARC, 1993
Rat/oral	35 days	No NOAEL; LOAEL 0.01 mg/kg-day	Male reproductive toxicity – impaired spermatogenesis	Ezekiel et al., 2011
	9 weeks	No NOAEL; LOAEL 0.015 mg/kg every other day		IARC, 2002
Rat/ gavage	14 days	No NOAEL; LOAEL 7.5 mg/kg-day	Female reproductive toxicity – decreased conception	Ibeh and Saxena, 1997a; Gupta,

Species/ Route	Duration	NOAEL/ LOAEL	Effect/Comments	Citation
				2011
			No clear NOAEL or LOAEL for developmental toxicity	
			Carcinogenic to humans – high confidence	

4.1.5 References

Agag, B.I. 2004. Mycotoxins in foods and feeds. 1-Aflatoxins. Ass Univ Bull Environ Res. 7(1), 173-206.

Agnes, V.F., Akbarsha, M.A. 2003. Spermatotoxic effect of aflatoxin B1 in the albino mouse. Food Chem Toxicol. 41, 119-130.

Butler, W.H. 1964. Acute toxicity of aflatoxin B₁ in rats. Br J Cancer. 18(4), 756-762.

Eaton, D.L., Groopman, J.D. (Eds.) 1994. The Toxicology of Aflatoxins: Human Health, Veterinary, and Agricultural Significance. Academic Press. San Diego, CA.

EFSA (European Food Safety Authority). 2013. Aflatoxins (sum of B1, B2, G1, G2) in cereals and cereal-derived food products. Technical Report. European Food Safety Authority. Parma, Italy.

EPA (U.S. Environmental Protection Agency). 2004. Guidance for Clinicians on the Recognition and Management of Health Effects related to Mold Exposures and Moisture Indoors. Center for Indoor Environments and Health, University of Connecticut. U.S. Environmental Protection Agency.

Ezekiel, C.N., Alabi, O.A., Anokwuru, C.P., Oginni, O. 2011. Studies on dietary aflatoxin-induced genotoxicity using two *in vivo* bioassays. Arch Appl Sci Res. 3(2), 97-106.

FDA (U.S. Food and Drug Administration). 2013. Aflatoxins. Bad Bug Book: Foodborne Pathogenic Microorganisms and Natural Toxins Handbook. U.S. Food and Drug Administration. Available at:
<http://www.fda.gov/Food/FoodborneIllnessContaminants/CausesOfIllnessBadBugBook/ucm071020.htm>

Guevara-González, R.G. 2011. Aflatoxins - Biochemistry and Molecular Biology. InTech, Rijeka, Croatia.

Gupta, R.C. 2011. Aflatoxins, ochratoxins and citrinin. Chapter 55. In: Gupta, R.C. (Ed.) Reproductive and Developmental Toxicology. Elsevier, Inc. p. 753-763.

Hsieh, D.P.H., Wong, J.J. 1994. Excretion of Aflatoxins. In: Eaton, D.L., Groopman, J.D. (Eds.) *The Toxicology of Aflatoxin. Human Health, Veterinary and Agricultural Significance*. New York, Academic Press, pp. 73-88. Available online at: https://books.google.com/books?id=6SAIBQAAQBAJ&pg=PA73&lpg=PA73&dq=excretion+of+aflatoxins&source=bl&ots=_C-EE3P9Ij&sig=OwV3O7e6yRgubYAWZUybe_y8874&hl=en&sa=X&ei=DKTbVPTGJMX_gwSk4YOACA&ved=0CB0Q6AEwADgK#v=onepage&q=excretion%20of%20aflatoxins&f=false

IARC (International Agency for Research on Cancer). 1993. Aflatoxins. IARC Monographs Vol. 56. International Agency for Research on Cancer. p. 246-395.

IARC (International Agency for Research on Cancer). 2002. Aflatoxins. IARC Monographs Vol. 82. International Agency for Research on Cancer. p. 171-300.

IARC (International Agency for Research on Cancer). 2012. Aflatoxins. IARC Monographs Vol. 100F. International Agency for Research on Cancer. p. 225-248.

Ibeh, I.N., Saxena, D.K. 1997a. Aflatoxin B₁ and reproduction. I. Reproductive performance in female rats. *Afr J Reprod Health*. 1(2), 79-84. Abstract only

Ibeh, I.N., Saxena, D.K. 1997b. Aflatoxin B₁ and reproduction. II. Game toxicity in female rats. *Afr J Reprod Health*. 1(2), 85-9. Abstract only

JECFA (Joint FAO/WHO Expert Committee on Food Additives). 2001. Safety evaluation of certain mycotoxins in food. Prepared for the fifth-sixth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) WHO Food Additive Series, No. 47/FAO Food and Nutrition paper 74. Food and Agriculture Organization. World Health Organization. Rome, Geneva.

Joffe, A.Z., Ungar, H. 1969. Cutaneous lesions produced by topical application of aflatoxin to rabbit skin. *J Invest Dermatol*. 52(6), 504-507.

Johnson, W., Guengerich, F.P. 1997. Reaction of AFB₁ exo-8,9-epoxide with DNA: Kinetic analysis of covalent binding and DNA-induced hydrolysis. *Proceed Nat Acad Sci*. 94(12), 6121-6125.

McKean, C., Tang, L., Tang, M., Billam, M., Wang, Z., Theodorakis, C.W., Kendall, R.J., Wang, J.S. 2006. Comparative acute and combinative toxicity of aflatoxin B₁ and fumonisin B₁ in animals and human cells. *Food Chem Toxicol*. 44, 868-876.

Mykkanen, H., Zhu, H., Salminen, E., Juvonen, R.O., Ling, W., Ma, J., Polychronaki, N., Kemilainen, H., Mykkanen, O., Salminen, S., El-Nezami, H. 2005. Fecal and urinary excretion of aflatoxin B₁ metabolites (AFQ₁, AFM₁, and AFB-N⁷-guanine) in young Chinese males. *Int J Cancer*. 115, 879-884.

Newberne, P.M., Russo, R., Wogan, G.N. 1966. Acute toxicity of aflatoxin B₁ in the dog. Path Vet. 3, 331-340.

Peraica, M., Radic, B., Lucic, A., Pavlovic, M. 1999. Toxic effects of mycotoxins in humans. Bull World Health Org. 77(9), 754-766.

Smerak, P., Barta, I., Polivkova, Z., Bartova, J., Sedmikova, M. 2001. Mutagenic effects of selected trichothecene mycotoxins and their combinations with aflatoxin B₁. Czech J Food Sci. 19, 90-96. Available at: <http://www.agriculturejournals.cz/publicFiles/84640.pdf>

Talebi, E., Khademi, M., Rastad, A. 2011. An over review of effect of aflatoxin in animal husbandry. Asian J Exp Biol Sci. 2(4), 754-757.

Williams, J.H., Phillips, T.D., Jolly, P.E., Stiles, J.K., Jolly, C.M., Aggarwal, D. 2004. Human aflatoxicosis in developing countries: a review of toxicology, exposure, potential health consequences, and interventions. Am J Clin Nutr. 80, 1106-1122.

4.2 Alternaria Mycotoxins

Alternaria species produce more than 70 mycotoxins and phytotoxins, but only a few are of toxicological significance (EFSA, 2008). Furthermore, only a small proportion have been chemically characterized and reported to cause health effects in humans and animals. *Alternaria* toxins are divided into different classes based on their chemical structures. The first class (dibenzo- α -pyrones) includes the complex heterocyclic phenolic compounds alternariol (AOH), alternariol monomethyl ether (AME), altenuene (ALT) and altenuisol (AS). The second class consists of perylene quinones, and includes altertoxins I, II, and III (ATX-I, ATX-II and ATX-III), as well as stemphytoxin III. The third class is the tetramic acids, which include tenuazonic acid (TeA) and iso-tenuazonic acid (iso-TeA). The fourth class is the AAL-toxins, which are ester derivatives. Miscellaneous structures including the cyclic tetrapeptide tentoxin (TEN) are the fifth class (EFSA, 2008, 2011).

4.2.1 Physical and Chemical Characteristics

The physical and chemical characteristic of only some of the *Alternaria* toxins are found in the literature. AOH, AME, ALT, and ATX-1 are solids with melting points ranging from 180⁰C to 350⁰C and are soluble in most organic solvents. AOH, AME and ALT are stable at pH 5 for at least 5 hours in water, but degraded in 0.1 M KOH (EFSA, 2011). ALT was also stable at pH 7, but information on stability of AOH or AME at pH 7 in aqueous solution was not provided by EFSA. Information on the stability of the other mycotoxins was not available.

4.2.2 Toxicokinetics

No *in vivo* toxicokinetic information on *Alternaria* toxins is available for humans, and only a single *in vivo* toxicokinetic study is available in experimental animals. The toxicokinetics of

AME was investigated in adult male rats given a single gavage dose of 0.25 mg/kg ¹⁴C-labelled AME. AME appeared to be poorly absorbed, with 81-87% of the dose excreted in the feces in the form of unmetabolized AME. Only 5-9% of the dose was found in the urine, in the form of uncharacterized polar metabolites excreted mostly on day 1. The level of radioactivity in tissues was very low, and the study was not designed to evaluate distribution at early time points (apparently no blood sampling or interim sacrifices were performed).

The results of the *in vivo* study are consistent with the conclusion that AME is poorly absorbed, however, the AME that *is* absorbed is extensively metabolized, based on the finding that the urinary label was primarily in the form of metabolites. Theoretically, it is possible that the high fecal excretion reflects biliary excretion. However, the preponderance of unmetabolized AME in the feces is not consistent with absorption and biliary excretion, in light of the extensive metabolism reflected in the urinary excretion and the *in vitro* data described below.

In a preliminary study, male rats with cannulated bile ducts were given AOH by gavage, and the AOH metabolites present in the bile were examined (Burkhardt et al., 2011). The four major catechol metabolites and their *O*-methyl ethers reported by the same authors as being formed by microsomal incubation systems and by liver slices were present in the bile.

In Caco-2 cells, an *in vitro* model for studying intestinal absorption, AME is poorly absorbed (consistent with the *in vivo* data), while AOH is extensively and rapidly absorbed.

The conclusion that absorbed AME is highly metabolized is supported by results from *in vitro* studies with rat, porcine and human hepatic microsomal systems containing NADPH (EFSA, 2011). In these systems, AME was metabolized to 7 oxidative metabolites and demethylated to AOH. AOH was also converted to oxidative metabolites in these systems, and ALT was converted to the corresponding catechol. Catechol metabolites were also seen when AOH or AME was incubated with precision-cut rat liver slices, which are more similar to *in vivo* conditions than are microsomes. EFSA (2011) noted that the oxidative metabolism of AME and AOH to catechols and hydroquinones may have toxicological significance. Presumably the same concern would apply to the catechol formed from ALT. Based on the specific isoforms of human cytochrome P450 (CYP), EFSA (2011) stated that significant extrahepatic hydroxylation would be expected, particularly in the lung and esophagus for AME and AOH and in the intestine for ALT. AME and AOH are converted *in vitro* to their glucuronide and sulfate conjugates (EFSA, 2011). Thus the *Alternaria* toxins AME and AOH appear to be oxidatively metabolized to a variety of metabolites that may then be subsequently conjugated.

In vitro studies with TEN also reported CYP-mediated metabolism. Incubation of TEN with rat liver microsomes/NADPH resulted in partial isomerization to iso-TEN, followed by oxidative *N*-demethylation. Small amounts of hydroxylation products were also observed but not further characterized (EFSA, 2011).

No relevant information is available on the absorption, distribution, excretion or metabolism for any of the other *Alternaria* toxins.

4.2.3 Hazard Information - Human

No epidemiology studies were reported that evaluated the potential association of *Alternaria* with any adverse or nonadverse effects (EFSA, 2011).

A. alternata contamination of grains is reported to be higher in regions with high esophageal cancer morbidity than in regions with low esophageal cancer morbidity. However, a definitive association with the mold and its mycotoxins is precluded, because the grains from counties of high incidence of esophageal cancer were also infected by other toxigenic fungi, such as *Penicillium cyclopodium*, *Fusarium moniliforme*, *Aspergillus nidulans* and *Aspergillus fumigates* (EFSA, 2011). However, no evidence was located for an association between *Penicillium* or *Aspergillus* and esophageal cancer; *Fusarium* was not evaluated for this report.

4.2.4 Hazard Information - Animal

4.2.4.1 Acute Lethality

EFSA (2011) reported LD₅₀ studies in rats and mice by oral and parenteral routes.

Oral LD₅₀ for the sodium salt of TeA were 225 mg/kg (male mice), 81 mg/kg (female mice), 180 mg/kg (male rats), and 146 mg/kg (female rats). The iv LD₅₀ were 162 mg/kg (male mice), 115 mg/kg (female mice), 146 mg/kg (male rats), and 157 mg/kg (female rats). In male ICR mice, TeA was administered by various routes (oral, subcutaneous -sc, ip, iv). The highest LD₅₀ was for the oral route (225 mg/kg) and the lowest LD₅₀ was for the iv route (125 mg/kg). Diarrhea, muscle tremor and convulsions were reported symptoms from these studies.

Of the dibenzo- α -pyrones group (ALT, AOH, AME), ALT was the most toxic, based on ip dosing of mice. For AOH and AME, the LD₅₀s in mice were greater than 400 mg/kg, while the ALT LD₅₀ was between 50 and 400 mg/kg. Signs of toxicity signs in mice dosed with AOH and AME included gastric spasms and periodic panting.

The ATX-II ip LD₅₀ was less than 200 mg/kg, while the ATX-I ip LD₅₀ was between 100 and 200 mg/kg. Administration of ATX-I and ATX-II caused subendocardial and subarachnoid hemorrhaging.

Lethality (degree not available) was reported in mice that received ip dosing with 100 mg/kg-day of partially purified crude *Alternaria* extracts for 3 consecutive days.

4.2.4.2 Acute/Short-term Toxicity

No data regarding primary eye or skin irritation or skin sensitization were reported in EFSA (2011).

In an oral study, female rats (groups of 4) were fed rodent chow with different *Alternaria* cultures that produced different ratios of AME, AOH, ALT and TeA. The *Alternaria* cultures were mixed in at either 10 or 50 % of the total ration and fed to rats ad libitum for 21 days (Sauer, 1978). No signs of toxicity were reported in the 10% diets. In the 50% diet, no toxicity was reported in the two groups with diets that did not contain TeA. Necropsy findings, including “examination of the reproductive tracts for estrogenic effects,” were negative, but additional details were not provided. The groups with TeA in the diet at 145 ppb and higher had signs of toxicity, including decreased food consumption, weight loss, and death. These groups also differed from the other groups in that they contained an undetermined amount of ATX-I. EFSA did not convert the ingested amount into a daily dose. In addition, since the two groups that received TeA also received ATX-I, it is not clear which mycotoxin was the toxic agent (or whether both were).

A single exposure of monkeys to TeA doses up to 50 mg/kg did not result in signs of toxicity, but emesis, diarrhea and gastrointestinal hemorrhage was reported at unspecified higher doses (EFSA, 2008). EFSA (2011) described a study in which one male and one female monkey were given oral doses of TeA at 22 mg/kg-day for 21 days, 48.8 mg/kg-day on study days 22-32, and 89.6 mg/kg-day on study day 33 through the end of the study. No clinical signs of toxicity were seen at the first two dose levels. At 89.6 mg/kg-day, both monkeys vomited. In addition, one animal had bloody diarrhea and become moribund after 2 days at the high dose. Necropsy of this animal showed hemorrhagic gastroenteropathy. The other monkey continued vomiting after treatment, but “tolerated the treatment” at the high dose for 15 days. The actual high dose is unknown, since the vomiting may have eliminated much of the TeA.

Dosing with 10 mg/kg-day TEA in gelatin capsules (4 divided doses/day) resulted in moribundity in two beagle dogs within 8-9 days of dosing (EFSA, 2011). Diarrhea, vomiting and hemorrhages in the lung and gastrointestinal tract were also reported, along with microscopic evidence of hemorrhage in other organs and degenerative changes in the liver. As for the monkey study, the actual dose is unknown, since the vomiting may have eliminated much of the TeA.

4.2.4.3 Repeated Dose Toxicity

Aside from the monkey study of TeA described under short-term toxicity, no repeated dose toxicity study was located for any *Alternaria* toxin.

4.2.4.4 Developmental and Reproductive Toxicity

The data on developmental toxicity of *Alternaria* toxins is very limited. No studies of reproductive toxicity were located, and only one study of developmental toxicity via an environmentally-relevant exposure route (an oral study of AME) was located. In addition, there are two studies of developmental toxicity in Syrian golden hamsters and mice following parenteral dosing (EFSA, 2011).

In the oral study (Pero et al., 1973), pregnant DBA/2 mice were administered 0 or 50 mg/kg-day AME by oral gavage in honey-water (1:1) on GD 9-12 or 13-16 and sacrificed on GD 20. Fetal survival, “runts” (not further defined), and malformations (visceral and skeletal) were reported. The authors reported no evidence of fetotoxicity and no statistically significant increase in malformed fetuses at the single dose tested. However, study reporting is limited, and the group sizes were small (4-14 dams, depending on dose and control).

In contrast, there was evidence of fetotoxicity at sufficiently high doses following parenteral administration (Pero et al., 1973). Administration of 50 mg/kg-day AOH to DBA/2 mice sc in dimethyl sulfoxide (DMSO) on gestation day (GD) 9-12 or GD 13-16 resulted in no signs of maternal or fetotoxicity. However, AOH administered sc at 100 mg/kg-day caused increased dead and resorbed fetuses/litter and increased runts/litter when administered on GD 9-12, and increased malformed fetuses/litter when administered on GD 13-16. AOH and AME (1:1) administered together to DBA/2 mice sc in DMSO, each at a dose of 25 mg/kg-day on GD 9-12, caused increased dead and resorbed fetuses and runts/litter. There was also a nonsignificant increase in malformed fetuses/litter. EFSA (2011) and the authors described this as a synergistic effect, but support for this conclusion is weak due to the study limitations (e.g., inconsistent controls for the single-chemical testing, small sample sizes, limited reporting).

Syrian golden hamsters were given a single ip dose of AME at 0 (not specified by EFSA, but implied), 50, 100, or 200 mg/kg on GD 8. At 200 mg/kg, AME was maternally toxic and fetotoxic (as demonstrated by increased resorptions and decreased fetal body weights) with no toxic effects reported at the lower doses.

4.2.4.5 Genotoxicity

There are no *in vivo* genotoxicity studies of *Alternaria* toxins.

AOH and AME were mostly positive in bacterial mutation assays with or without metabolic activation, acting at AT base pairs and causing frameshift mutations. ATX-I, ATX-II and ATX-III were also mutagenic with or without metabolic activation. The most potent of these, ATX-III, had potency in the *Salmonella* assay only 10-fold lower than that of aflatoxin B1, which is a potent mutagen and known human hepatocarcinogen. TeA and TEN were reported as nonmutagenic with or without metabolic activation.

An extract of *A. alternata* induced chromosome aberrations in human lymphocytes and mutations in Chinese hamster V79 cells, and cell transformation in mouse fibroblast cells. An extract of *A. alternata* induced unscheduled DNA synthesis (UDS, a measure of DNA damage) and sister chromatid exchanges.

Other studies investigated specific *Alternaria* mycotoxins, as opposed to mold extracts. AME isolated from the extract induced mutations in V79 cells and transformation of mouse fibroblasts. In another study, AOH caused significant increases in gene mutations in V79 cells and mouse lymphoma cells (and chromosome breakage). Increases in small colonies in the mouse lymphoma assay indicated the presence of large chromosomal deletions, consistent with other studies showing the induction of micronuclei by AOH. AME and AOH also induced mutations of the Ha-ras gene in cultured human fetal esophageal epithelium.

Nitrosylated ATX-I was mutagenic in V79 cells. ATX-I and ATX-III also induced transformation of mouse fibroblasts.

AOH and AME induced single strand DNA breaks in a variety of mammalian cell systems. It appears that glucuronidation eliminates the DNA strand breaking potential of these compounds. AOH is an inhibitor of DNA topoisomerase, and it has been proposed that at least some of its genotoxicity can be attributed to this inhibition. Thus, the gene mutation studies with AME and AOH are supported by studies showing that this class of mycotoxins can damage DNA.

EFSA (2011) concluded that “the *in vitro* data provide clear evidence of the genotoxicity of some *Alternaria* toxins in bacteria and mammalian cells. Mutagenic and clastogenic events, induction of DNA breaks as well as a potential role in cell transformation have been described for AOH and AME in several mammalian cell systems. Inhibition of DNA topoisomerases, which are necessary for normal functioning of the cells as well as production of oxidative damage, might account for these effects. If AOH genotoxicity is confirmed to be mostly exerted via inhibition of DNA topoisomerases... this indirect mechanism would involve the concept of a threshold for the genotoxic effects.” However, as noted, there is evidence that AOH also induced point mutations, which would not be due to an effect on topoisomerase.

4.2.4.6 Carcinogenicity

Minimal data are available on the carcinogenic properties of the *Alternaria* toxins. Yekeler et al. (2001) dosed male Swiss albino mice (10 animals/group) with 0, 50, or 100 mg/kg-day of AME, or 25 mg/kg-day of TeA in the drinking water for 10 months. The concentration in drinking water was adjusted based on the water consumption to maintain constant dosing. Body weights were measured, and the histology of the esophagus was examined. Body weights were significantly lower than controls in the 100 mg/kg-day AME and 25 mg/kg-day TeA treatment groups. Precancerous changes ranging from mild dysplasia to severe dysplasia were reported in the esophageal mucosa. In control animals, no precursor lesions were detected. The highest

number of precursor lesions on esophageal mucosa were reported in mice treated with TeA (25 mg/kg-day) followed by AME at 100 mg/kg-day and then AME at 50 mg/kg-day. These changes were statistically significant when compared to controls. EFSA (2011) reported that the interpretation of histopathology figures of esophageal lesions was considered questionable by the CONTAM panel, which was “not fully convinced by the presence of dysplastic changes in the (o)esophageal mucosa.” This study is not adequate for evaluation of carcinogenicity of any other tissue, due to the small sample size and short exposure duration.

A non-standard *in vitro/in vivo* study provided some evidence of AOH carcinogenicity. Tissue blocks of the esophagus of human embryo were cultured for 1 week, treated with AOH for 24 hours, and then cultured for 2 more weeks. Nude mice (BALB/c) were implanted with these treated cultures, and squamous cell carcinoma developed in 1 of the 3 animals (EFSA, 2011).

4.2.4.7 In Vitro Studies and Data Related to Mode of Action

The mechanism of toxicity for the *Alternaria* toxins is agent specific and not well characterized (EFSA, 2011). AAL-toxins are structurally related to sphingoid bases, which play important roles in cell communication and signal transduction. AAL-toxin toxicity is believed to result primarily from disruption of sphingolipid biosynthesis. AAL-toxins increased sphinganine concentration in rat primary hepatocytes, perhaps as a result of feedback from decreased sphingolipid levels. Sphingolipids are structural components of cell membranes and play a role in proliferation and cell death. Consistent with the latter role of sphingolipids, apoptosis was induced by AAL-TA1 in African green monkey kidney cells.

The mechanism of cytotoxicity for TeA is reported to be the inhibition of protein synthesis by inhibiting protein release from the ribosomes.

AOH is structurally similar to some estrogen-like compounds. It bound to cell-free recombinant human estrogen receptor and functioned in some assays as a weak estrogen receptor antagonist, although results differed in different test systems. AOH and AME, but not TeA, decreased progesterone synthesis in porcine ovarian cells *in vitro* (EFSA, 2011). These data suggest that AOH and AME may have reproductive effects, but the relevance of these findings is unclear in the absence of dose-response data at relevant doses via relevant routes.

Table 7. Summary of *Alternaria* Toxin Toxicity Data

Study Type	Route, Species (duration)	References
Toxicokinetics	1 oral study (AME) in rats, <i>in vitro</i> studies (AME, AOH, ALT, TEN)	EFSA, 2011
Acute lethality	4 oral studies (TeA), 2 in mice and rats (LD ₅₀ ranged	EFSA, 2008 EFSA, 2011

Study Type	Route, Species (duration)	References
	from 81-225 mg/kg; 3 parenteral studies (TeA) in mice and rats with LD ₅₀ range of 81-150 mg/kg 2 (ip) injection studies in mice (AOH, AME, AOH + AME, ALTATX-I, and ATX-II); (in general 50 < LD ₅₀ < 400 mg/kg) 1 study of <i>Alternaria</i> extracts in mice	
Acute/short-term toxicity	1 dietary study in rats for 21 days, (varying mixtures of AME, AOH, ALT, TeA, ATX-I); 2 oral study in monkeys, one for 1 day, one for > 33 days (TeA); 1 oral study in dogs for <10 days (TeA)	EFSA, 2011
Primary Irritation	No data	
Sensitization	No data	
Repeated dose toxicity	No data	EFSA, 2011
Developmental	1 oral gavage study in honey:water from GD 9-12 or 13-16 (AME), 1 sc study in DMSO on GD 9-12 or 13-16 (AOH and/or AME); 1 ip study in Syrian golden hamsters on GD 8 (AME)	Pero et al., 1973; EFSA, 2011
Reproductive	No <i>in vivo</i> data	
Genotoxicity	6 bacterial mutation assays with AME and/or AOH; 3 bacterial mutation assays with ATX-I, ATX-II, and/or ATX-III; 1 bacterial mutation assay with TeA and TEN; multiple DNA damage assays with AOH and/or AME; 2 chromosome damage assays with AOH and/or AME; 3 studies of mammalian gene mutation with AOH and/or AME; 2 studies of cell transformation and/or mammalian gene mutation with ATX-I and/or ATX-III. <i>Alternaria</i> extract positive for DNA damage, mammalian gene mutation, and chromosome damage	
Carcinogenicity	1 dietary study in mice for 10 months (AME or TeA)	Yekeler et al. 2001

4.2.4.8 Lowest Hazard Endpoints

The data on the *Alternaria* toxins are extremely limited and insufficient to definitively identify targets of toxicity or effect levels. Therefore, no table of effect levels is provided. However, a small number of parenteral studies suggest that AOH and AME may cause developmental effects, but this suggestion has not been evaluated following environmentally-relevant exposure routes. AOH might also cause reproductive effects, based on its structural resemblance to

estrogen, but this possibility has not been tested *in vivo*. Limited data also suggest that TeA affects the gastrointestinal tract and causes hemorrhaging in the lung, gastrointestinal tract, and other organs. AME and AOH also had “convincing” (EFSA, 2011) evidence of *in vitro* genotoxicity, causing both gene mutations and chromosome aberrations. Less extensive data also suggest that the ATX compounds are mutagenic and cause cell transformation. There are limited data suggesting that AME and possibly TeA are carcinogenic, but the data are far from convincing.

Due to the absence of adequate data on effect levels, EFSA (2011) used a threshold of toxicological concern (TTC) approach in its risk assessment of *Alternaria* toxins, as genotoxic chemicals, based on the genotoxicity of AOH and AME.

4.2.5 References

Burkhardt, B., Wittenauer, J., Pfeiffer, E., Schauer, U.M.D., Metzler, M. 2011. Oxidative metabolism of the mycotoxins alternariol and alternariol-9-methyl ether in precision-cut rat liver slices *in vitro*. *Mol. Nutr. Food Res.* 55, 1079–1086.

EFSA (European Food Safety Authority). 2008. Scientific information on mycotoxins and natural plant toxicants. CFP/EFSA/CONTAM/2008/01. European Food Safety Authority.

EFSA (European Food Safety Authority). 2011. Scientific Opinion on the risks for animal and public health related to the presence of *Alternaria* toxins in feed and food. *EFSA Journal* 9(10), 2407.

Pero, R.W., Posner, H., Blois, M., Harvan, D., Spalding, J.W. 1973. Toxicity of metabolites produced by the *Alternaria*. *Environ. Health Persp.* 4, 87-94.

Sauer, D.B., Seitz, L.M., Burroughs, R., Mohr, H.E., West, J.L., Milleret, R.J., Anthony, H. D. 1978. Toxicity of *Alternaria* metabolites found in weathered sorghum grain at harvest. *J. Agric. Food Chem.* 26(6), 1380-1389.

Yekeler, H., Bitmis, K., Ozcelik, N., Doymaz, M.Z., Calta, M. 2001. Analysis of toxic effects of *Alternaria* toxins on esophagus of mice by light and electron. *Toxicol. Pathol.* 29(4), 492-497

4.3 Chaetomium Toxins

The *Chaetomium* fungi produce a large number of bioactive metabolites. A recent study conducted a careful screen of the metabolites formed from 25 strains of *C. globosum* isolated from building materials, and identified the major compounds as chaetoglobosin A, C and F, chaetomugilin D, and chaetoviridin A (McMullin et al., 2013). Minor metabolites included

chaetomugilin I, chaetoviridin E, azaphilones, and other chaetoglobosins. Other strains have been reported to produce chetomin, chaetocin, and cochliodinol, but McMullin et al. (2013) suggested that the species being investigated may have been misidentified in some studies. Chaetochromin is a polyphenolic compound that is reported to be isolated from several *Chaetomium* species (Ito and Ohtsubo, 1987). Other metabolites that have been identified include epipolythiodioxopiperazines, xanthenes, anthraquinones, chromones, depsidones, terpenoids, and steroids (Zhang et al., 2012). Although many of these compounds are bioactive (e.g., antitumor, antibiotic, cytotoxic activity), most are not well characterized, and their relevance to human exposure to mold in consumer products is unknown.

4.3.1 Physical and Chemical Characteristics

Only some of the physical and chemical characteristics of the *Chaetomium* toxins are found in the literature (McMullin et al., 2013). The chaetoglobosins are structurally related to cytochalasans.

Table 8. Physical and Chemical Characteristics of Chaetomium Toxins

Characteristic		Reference
<i>Chaetoglobosin A</i>		McMullin et al., 2013
Molecular formula	$C_{32}H_{36}N_2O_5$	
Molecular weight	529.27	
Physical State	pale yellow solid	
<i>Chaetoglobosin F</i>		McMullin et al., 2013
Molecular formula	$C_{32}H_{38}N_2O_5$	
Molecular weight	531.28	
Physical State	pale yellow solid	
<i>Chaetoglobosin C</i>		McMullin et al., 2013
Molecular formula	$C_{32}H_{38}N_2O_5$	
Molecular weight	529.27	
Physical State	colorless solid	
<i>Chaetomugilin D</i>		McMullin et al., 2013
Molecular formula	$C_{23}H_{27}O_6Cl$	
Molecular weight	435.16	
Physical State	yellow gum	
<i>Chaetoviridin A</i>		McMullin et al., 2013
Molecular formula	$C_{23}H_{25}O_6Cl$	
Molecular weight	433.14	
Physical State	yellow gum	

4.3.2 Toxicokinetics

No *in vivo* toxicokinetic information on *Chaetomium* toxins is available for humans or experimental animals. However, the report by Ohtsubo et al. (1978) showing much higher toxicity following parenteral exposure compared to oral dosing of the same strains of rats and mice, suggests low oral absorption or first pass metabolism. No *in vitro* data on metabolic pathways was located.

4.3.3 Hazard Information - Human

No epidemiology or clinical studies were found that evaluated the potential toxicity of *Chaetomium* toxins.

4.3.4 Hazard Information - Animal

4.3.4.1 Acute Lethality

The only available acute lethality studies are in a single publication investigating the toxicity of chaetoglobosin A by a variety of routes. These studies found high toxicity and rapid death following parenteral injection, but much lower toxicity via oral exposure. The oral administration of a single dose of 50 to 400 mg/kg chaetoglobosin A in olive oil had minimal effect on mice and rats (Ohtsubo et al., 1978). The only effect noted at 400 mg/kg was a transient body weight loss in both rats and mice.

In Wistar rats, ip injection of chaetoglobosin A (dissolved in DMSO) at 2-16 mg/kg caused lethality within 10 minutes to 2 hours. A post-mortem effect noted for these rats was visceral congestion. The LD₁₀₀ was 2 mg/kg rats (Ohtsubo et al., 1978).

In mice (DDD strain) injected sc, LD₅₀ values for chaetoglobosin A were estimated at 6.5 and 17.8 mg/kg for male and female, respectively. Toxic effects noted included hypokinesia, coldness and edematous swelling at the site of injection. Sc injection of 5 mg/kg of chaetoglobosin A killed 5/14 mice within 6-48 hours. Toxic effects noted included pulmonary congestion, scattered necrosis of the spleen and depletion of thymocytes, as well as spermatocyte degeneration. After 3 days of recovery, only effects on the thymus and testes were noted, and no histological abnormalities were seen after 7 days of recovery (Ohtsubo et al., 1978).

4.3.4.2 Acute/Short-term Toxicity

No information is reported

4.3.4.3 Repeated Dose Toxicity

No studies on repeated dosing of any *Chaetomium* toxin was located, but administration of a rice culture of *C. globosum* at a concentration of 50% in feed to rats and mice for 10-15 days resulted only in decreased body weight gain (Ohtsubo et al., 1978). No information on toxin levels was

available. Delayed liver injuries, bone marrow aplasia, and atrophy of lymphatic tissue were reported in DDD mice dosed with 30 ppm chaetochromin in feed (about 6 mg/kg-day, based on a food factor of 0.2) for 2 weeks (Ito and Ohtsubo, 1987). The primary study reports provided few details.

4.3.4.4 Developmental and Reproductive Toxicity

The data on developmental toxicity of *Chaetomium* toxins is limited to one study in ICR mice (Ito and Ohtsubo, 1987). Pregnant mice were fed a diet containing moldy rice that provided 0, 10 or 30 ppm of chaetochromin on GD 0-18 (both doses), 7-9 (30 ppm), or 10-12 (30 ppm). Based on a food factor of 0.2, the doses were approximately 0, 2, and 6 mg/kg-day. There was no maternal toxicity, based on changes in body weight, organ weight, or histological findings, although there was an increase in mitotic figures in the liver at both doses. Fetal data were reported only as average results, not in the more appropriate form of per litter analyses. Statistically significant decreases in living fetuses and increased resorptions were seen at the high dose. Malformations, including exencephaly were observed in 10 of the 97 fetuses on the diet containing 30 ppm of the fungal toxin. No effects were observed in the litters exposed on GD 7-9 or 10-12, even though the critical period for exencephaly is GD 7-8. There was also a dose-related decrease in fetal body weight at both doses. The study authors noted that maternal toxicity in this strain was much lower than toxicity in the DDD mouse strain.

4.3.4.5 Genotoxicity

Very limited information was found on the mutagenicity of the *Chaetomium* toxins. Umeda et al. (1977) evaluated the mutagenicity of chaetoglobosin B in FM3A cells, a C3H mouse mammary carcinoma cell line, based on induction of 8-aza guanine resistance. There was no clear positive response, although there was a slight increase in mutation frequency.

4.3.4.6 Carcinogenicity

No information was reported in the literature reviewed.

4.3.4.7 In Vitro Studies and Data Related to Mode of Action

Cytochalasins and chaetoglobosins are reported to exhibit diverse *in vitro* effects including inhibition of postmitotic cytoplasmic cleavage of HeLa cells, inhibition of cell movement, and nuclear extraction (Ohtsubo et al., 1978).

The cytotoxicity of the *Chaetomium* toxins was investigated with KB cells (Koyama et al., 1988). ED₅₀ values of approximately 0.050 µg/mL were reported. Isolates from 14 different *Chaetomium* strains inhibited ciliary beating in a chick tracheal organ culture (Pieckova, 2003), but the significance of this observation is unclear in the absence of reported doses.

Chaetoglobosin F was reported to alter maturation and immune responses of bone marrow derived dendritic cells. It inhibited the activation of the TLR4 pathway controlling the formation of cytokines and surface molecules. The results suggest chaetoglobosin F may act as an immunosuppressor at sufficiently high doses (Hua et al. 2013).

Table 9. Summary of Toxicity Data of Chaetomium Toxins

Toxin/Study Type	Route, Species (duration)	References
Chaetoglobosin A/Acute lethality	1 oral study in mice, 1 oral study in rats (LD ₅₀ > 400 mg/kg) 1 sc study in rats and 1 in mice (LD ₅₀ 6.5 male-17.8 female mg/kg) 1 ip study in rats (LD ₁₀₀ = 2 mg/kg)	Ohtsubo et al., 1978
Chaetochromin/Developmental toxicity	1 oral study in mice	Ito and Ohtsubo, 1987
Chaetoglobosin B/Genotoxicity	1 gene mutation study in mammalian cells	Umeda et al., 1977

4.3.4.8 Lowest Hazard Endpoints

The data on the *Chaetomium* toxins are extremely limited and insufficient to identify targets of toxicity or effect levels. In addition, insufficient data are available to make generalizations by class of toxin. Therefore, no effect level table is included. However, a single incompletely reported study suggests that chaetochromin may cause developmental effects. There are also inconsistent reports of liver and immunotoxicity with chaetochromin, with differences observed across mouse strains. The limited data available on chaetoglobosin A suggests that its oral toxicity may be low.

No risk assessments of *Chaetomium* toxins were located.

4.3.5 References

Hua, C., Yang, Y., Sun, L., Dou, H., Tan, R., Hou, Y. 2013. Chaetoglobosin F, a small molecule compound, possesses immunomodulatory properties on bone marrow-derived dendritic cells via TLR9 signaling pathway. *Immunobiology*. 218, 292-302.

Ito, Y., Ohtsubo, K. 1987. Teratogenicity of oral Chaetochromin, a polyphenolic mycotoxin produced by *Chaetomium* spp., to mice embryo. *Bull Environ Contam Toxicol*. 39, 299-303.

Koyama, K., Ominato, K., Natori, S., Tashiro, T., Tsuruo, T. 1988. Cytotoxicity and antitumor activities of fungal bis(naphtho- γ -pyrone) derivatives. *J Pharmacobio-Dyn*. 11, 630-635.

McMullin, D.R., Sumarah, M.W., Miller, J.D. 2013. Chaetoglobosins and azaphilones produced by Canadian strains of *Chaetomium globosum* isolated from the indoor environment. *Mycotoxin Res.* 29, 47-54.

Ohtsubo, K., Saito, M., Sekita, S., Yoshihira, K., Natori, S. 1978. Acute toxic effects of Chaetoglobosin A, a new cytochalasan compound produced by *Chaetomium globosum*, on mice and rats. *Japan J Exp Med.* 48(2), 105-110.

Pieckova, E. 2003. In vitro toxicity of indoor *Chaetomium* Kunze ex Fr. *Ann Agric Environ Med.* 10, 9-14.

Umeda, M., Tsutsui, T., Saito, M. 1977. Mutagenicity and inducibility of DNA single-strand breaks and chromosome aberrations by various mycotoxins. *Gann.* 68, 619-625.

Zhang, Q., Li, H.Q., Zong, S.C., Gao, J.M., Zhang, A.L. 2012. Chemical and bioactive diversities of the genus *Chaetomium* secondary metabolites. *Mini-Rev Med Chem.* 12, 127-148.

4.4 Ciclosporin/Cyclosporine

Cyclosporine (also called cyclosporine A) can be synthesized from the fungus *Tolypocladium inflatum*, also known as the cyclosporin fungus, as well as by members of the genus *Stachybotrys*. Cyclosporine is also manufactured and produced by several US pharmaceutical companies and is FDA approved (NTP, 2014). Cyclosporine is used clinically as an immunosuppressant, and so data are available from both extensive animal toxicity testing and controlled human exposure. Other cyclosporins also exist, but are not addressed in this report.

4.4.1 Physical and Chemical Characteristics

Table 10. Physical and Chemical Characteristics of Cyclosporine

Characteristic		Reference
Molecular formula	C ₆₂ -H ₁₁₁ -N ₁₁ -O ₁₂	ChemIDplus
IUPAC	30-Ethyl-33-[(E)-1-hydroxy-2-methylhex-4-enyl]-1,4,7,10,12,15,19,25,28-nonamethyl-6,9,18,24-tetrakis(2-methylpropyl)-3,21-di(propan-2-yl) 1,4,7,10,13,16,19,22,25,28,31-undecazacyclotritriacontane-2,5,8,11,14,17,20,23,26,29,32-undecone	IARC, 2012
CAS No	59865-13-3	ChemIDplus
Molecular weight	1202.6	ChemIDplus
Melting Point	148-151 deg C	ChemIDplus

Characteristic		Reference
Physical State	white or essentially white, fine crystalline powder	IARC, 2012
Solubility	Slightly soluble in water and saturated hydrocarbons; very soluble in methanol, acetone, and diethyl ether	HSDB, 2009

4.4.2 Toxicokinetics

Extensive information in humans is available on the toxicokinetics of cyclosporine and its various drug formulations (IARC, 1990; PDR, 2015), although it should be recognized that the specific formulation can also affect toxicokinetics. Cyclosporine is readily absorbed and widely distributed in both animals and humans (Ryffel and Mihatsch, 1986; IARC, 1990). In human blood, cyclosporine is extensively distributed in erythrocytes (Tedesco and Haragsim, 2012). Approximately 90% of circulating cyclosporine is bound to plasma proteins (PDR, 2015). Bioavailability after an oral dose reflects inter-individual variability in intestinal absorption, which is affected by factors, such as food ingestion, disease, stomach problems, and diarrhea. Absorption of pharmacologic doses and formulations of cyclosporine has been reported to range from 10% to 90%, largely varying with the patient population (e.g., liver vs. renal transplant patients). The peak blood concentration of cyclosporine following oral pharmacologic dosing occurs between 1 and 8 hours. The distribution half-life was reported to vary from 0.1 to 0.5 hours following iv dosing. The immunosuppressive effects of cyclosporine are attributed to the parent compound. Metabolism in humans is primarily via the hepatic cytochrome P450 system, followed by biliary excretion with a half-life of 6.4 to 8.4 hours. Hydroxylation via the P450 system is followed by secondary metabolism, either to further oxidation products or via demethylation of the primary metabolites. The expression of specific isoforms of P450 (CYP3A4 and CYP3A5) appear to play a role in the variability of cyclosporine metabolism. Cyclosporine is extensively metabolized, with more than 25 metabolites identified in human bile (IARC, 1990). Elimination is primarily via the feces.

Cyclosporine given orally to dogs and rats is absorbed and widely distributed throughout the animal. Approximately 50% of the dose was absorbed by beagle dogs and OFA or Wistar rats (Ryffel et al., 1983). In another study, approximately 30% of an oral dose was absorbed by Sprague-Dawley and Wistar rats. In rats, slow elimination occurred and even after 5 days significant amounts were observed. In rats, rabbits and dogs, cyclosporine is extensively metabolized by the P450 enzymes that results in hydroxylation and demethylation of cyclosporine (IARC, 1990). There was no evidence of accumulation in dogs following repeated dosing for a year (Ryffel et al., 1983).

4.4.3 Hazard Information – Human

Cyclosporine is classified as “Known to be a Human Carcinogen” (NTP, 2014); IARC (2012) stated that cyclosporine is “carcinogenic to humans (Group 1)”. Epidemiological studies show that cyclosporine is carcinogenic in humans, causing tumors at an incidence of less than 5% in the patient population (IARC, 1990). In particular, patients using cyclosporine have an increased chance of developing lymphoma, Kaposi sarcoma, and skin cancer (NTP, 2014), as well as cancers at other sites (IARC, 2012). Time to development of the tumor ranged from 1 month to 10 years (NTP, 2014). The most likely mode of action for the observed carcinogenicity is considered to be immunosuppression (NTP, 2014), but as noted below, it is likely that cyclosporine also acts via additional mechanisms.

Cyclosporine is a potent immunosuppressant, and is used to prevent organ and tissue rejection following transplantation. Oral cyclosporine is also used to treat severe rheumatoid arthritis, severe recalcitrant plaque psoriasis, atopic dermatitis, and is used in ophthalmic emulsions to increase tear production in adults whose tear production is suppressed (IARC, 2012).

Dosing and length of time for treatment depend on the type of treatment, with transplant patients receiving higher doses than other therapeutic applications. For example in patients being treated for rheumatoid arthritis, the initial dosing is 1.25 mg/kg twice daily (i.e., 2.5 mg/kg-day) for 4 weeks, whereas oral dosages for immunosuppression in newly transplanted patients start at 7 ± 3 to 9 ± 3 mg/kg-day, depending on the organ that is transplanted, and can last up to one year. For patients with kidney dysfunction or disease, the dose is much lower, starting with 2.5 mg/kg-day (PDR, 2015). Cyclosporine is usually given orally, but in bone marrow transplantation cyclosporine is given iv at 5-6 mg/kg daily for 3 months, followed by 12.5 mg/kg-day orally for 3-6 months (IARC, 2012). While oral and iv administration are most common, cyclosporine formulations are also available for parenteral, rectal, ophthalmic and pulmonary aerosol administration (Ragab et al., 2013). These doses for typical treatments (at which varying levels of immunosuppression occur) provide perspective on the doses at which adverse side effects are reported.

Nephrotoxicity is one of the most frequent toxic side effects observed following therapeutic dosing with cyclosporine. Cyclosporine as an immunosuppressive drug has a narrow therapeutic index (Da Silva et al., 2014) relative to the induction of nephrotoxicity. Both acute and chronic nephrotoxicity are associated with cyclosporine treatment. Acute nephrotoxicity caused by cyclosporine is characterized by renal vasoconstriction and renal dysfunction, and is reversible with discontinuation or reduction of the cyclosporine dose. Chronic nephrotoxicity, on the other hand, is irreversible and involves serious structure damage such as arteriopathy and tubulointerstitial fibrosis (Lee, 2010). Kidney transplant patients have a particularly high risk of chronic kidney toxicity following long term treatment with cyclosporine (Lee, 2010; Tedesco and Haragsim, 2012).

Other toxic effects reported in association with cyclosporine treatment include hepatotoxicity, neurotoxicity, hypertension, hyperlipidemia, gingival hyperplasia, hyperkalemia, hypomagnesaemia, hyperuricemia, and thrombotic microangiopathy (Da Silva et al., 2014; Tedesco and Haragsim, 2012). Several of these effects are likely associated with impaired kidney function, and these effects in general are consistent with the animal observations described below. PDR (2015) reported the following effects at therapeutic doses: hypertension, headache, influenza-like symptoms, diarrhea, nausea and vomiting, hirsutism, hypertrichosis, tremor, gum hyperplasia, hypertriglyceridemia, paresthesia, hyperesthesia, While many of these effects are also generally consistent with what is known about effects of cyclosporine, it is also important to note that the PDR (Physician's Desk Reference) captures all reported "adverse events," without a critical evaluation of causality.

4.4.4 Hazard Information - Animal

Ryffel et al. (1983) described many of the key animal toxicity studies. Although it is a primary reference, it provides much less detail than is typically standard for such studies.

4.4.4.1 Acute Lethality

Ryffel et al. (1983) reported LD₅₀ studies in several species by oral and parenteral routes. Oral LD₅₀s in mice rats and rabbits were >1000-2329 mg/kg. The corresponding range of iv LD₅₀s was >10 to 107 mg/kg. Adverse effects noted include hyperventilation, drowsiness, muscular spasms, weight loss and diarrhea.

4.4.4.2 Acute Short-term Toxicity

No data regarding primary skin irritation or sensitization were located.

One short term **oral** toxicity study was performed by Ryffel and Mihatsch (1986). Cyclosporine was given orally (manner of administration not specified) at 0 or 50 mg/kg-day for 14 days to spontaneously hypertensive (SH), Sprague-Dawley (SD), Wistar (WSA), Fischer (FIS), and Lewis (LEW) rats. Nephrotoxic effects noted in all strains included a reduction of kidney glomerular filtration rate as well as renal tubular changes; the order of sensitivity was the following: SH > FIS = LEW > WSA > SD.

Sprague-Dawley rats gavaged with 50 or 100 mg/kg per 48 hours (i.e., average of 25 or 50 mg/kg-day) for 21 days had reversible alterations in kidney (e.g., elevated serum urea and creatinine levels) and liver function; mild kidney histopathology (vacuolization of the proximal tubule, cell necrosis) was also reported (IARC, 1990). Administration of 20 or 40 mg/kg-day cyclosporine to an unspecified strain of rats in the diet for an unspecified period resulted in damage to the proximal nephron and proximal tubule. Similar effects were seen in other oral rat studies, but IARC (1990) did not provide additional details.

BALB/c mice were exposed to cyclosporine by **sc injection** of 200 mg/kg-day. The median survival time was 13 days with toxicity observed in kidneys, thymus, lymph nodes, spleen and the liver (IARC, 1990).

Cyclosporine was administered iv for four weeks to CD rats at 0, 6, 24, or 72 mg/kg-day and Cynomolgus monkeys at 0, 5, 25, or 75 mg/kg-day. In both of these studies, the highest dose was reduced (to 48 and 45 mg/kg-day, respectively) due to serious toxic effects, including mortality. At the two highest doses, adverse effects in both species included degenerative changes in the kidney and liver, changes in serum chemistry consistent with the liver and kidney effects, decreases in red blood cell markers but not white blood cells, marked neurological effects (sedation, ataxia), and atrophy of lymphoid tissue (Ryffel et al., 1983).

4.4.4.3 Repeated Dose Toxicity

For **13 weeks** OFA rats (10 males/10 females) were **fed** a dietary mixture with cyclosporine at 0, 14, 45, or 90 mg/kg-day, and Rhesus monkeys were orally dosed with cyclosporine using tragacanth (a gummy material – presumably used to prepare a gel-like lozenge) at 0, 20, 60, or 200 mg/kg-day (Ryffel et al., 1983). These studies evaluated body weight, food consumption, hematology and blood chemistry, and organ weight and histopathology. In rats, the two higher doses caused atrophy of lymphoid tissues and clear nephro- and hepatotoxicity. Gingivitis and atrophy of periodontal tissues was also noted. In monkeys, cyclosporine was well tolerated with minimal toxicity, and so the high dose was increased at 4 weeks to 300 mg/kg-day. Even at high doses, there were only minor functional and histopathological changes (transient decrease in leukocyte counts, elevated serum glutamyl phosphotransferase (SGPT) at high dose, variable atrophy of lymphatic organs).

Beagle dogs (4 males/4 females per group) were administered cyclosporine by gavage in olive oil at 0, 5, 15, or 45 mg/kg-day for one year (Ryffel et al., 1983). Reversible hypertrophic gingivitis with mononuclear cell infiltration and atypical cutaneous papillomatosis occurred at 45 mg/kg-day. Other effects, including anemia, leucopenia and thrombocytosis, were attributed to malnutrition or stress. There was no evidence of hepatotoxic or nephrotoxic effects.

Outbred New Zealand White (NZW) and Red Burgundy (RB) adult rabbits were treated subcutaneously with cyclosporine at 15 mg/kg-day for 60 days (Gratwohl et al., 1986). This study demonstrates a unique toxic syndrome in rabbits that is characterized by weight loss, reduced food and water consumption, and reduced movement. Dose dependent mortality was observed within 60 days of treatment, and animals had distended stomachs and intestines (Gratwohl et al., 1986). No evidence of nephrotoxicity was determined upon histological analysis of the kidneys.

Other repeated dose toxicity studies are summarized in the context of the cancer assessment, below.

4.4.4.4 *Developmental and Reproductive Toxicity*

Developmental toxicity was evaluated in OFA rats and GESI rabbits by Ryffel et al. (1983). Cyclosporine was given to pregnant female **rats** by oral administration in 2% gelatin at 0, 10, 17, 30, 100 or 300 mg/kg-day (30/group except for two high doses with 10/group) on postcoital days 6-15, and the rats were sacrificed on day 21. At doses up to 10 mg/kg-day there was no embryo toxicity (based on postimplantation loss, litter size, morphology, or fetal weight). Cyclosporine at 17 mg/kg-day resulted in a statistically significant increase in postimplantation loss (apparently on a pup basis, not the more appropriate litter basis), and 30 mg/kg-day was toxic to both dams and offspring. Maternal body weight gain was decreased by 50% at 30 mg/kg-day, accompanied by 90% postimplantation loss, lower fetal weights, and increased skeletal retardations. In a rabbit study, cyclosporine was given orally in 2 % gelatin at 0, 10, 30, 100 or 300 mg/kg-day on postcoitum day 6-18, and the rabbits were sacrificed on day 29, after delivery. In dams, 100 mg/kg was toxic, causing a decrease in weight gain. Fetal effects (all at 100 mg/kg-day) included increased post-implantation loss, decreased mean body weights and 24 hour survival, and increased skeletal retardation. Thus, clear developmental toxicity was seen only at a maternally toxic dose (30 mg/kg-day in rats, 100 mg/kg-day in rabbits). Postimplantation loss was also increased in rats at 17 mg/kg-day, but the data were presented only on a per pup basis.

Two additional studies showed fetotoxicity in rats at late gestational stages at a maternally toxic dose (25 mg/kg-day); evidence of kidney toxicity in the fetuses was also reported (IARC, 1990).

Fertility was examined in male (15/group) and female (30/group) Wistar rats treated with oral doses (manner of administration not specified) of cyclosporine in 2% gelatin at 0, 1.5, 5, or 15 mg/kg-day (Ryffel et al., 1983). Prior to mating, male rats were treated for 12 weeks, and female rats were treated for two weeks; treatment of females continued until weaning of offspring. Clinical observations and body weights were assessed in both sexes. Maternal endpoints included prenatal and postnatal copulation and pregnancy rates, the mean time to mating (precoital intervals), and pregnancy lengths. Evaluations of embryos examined prenatal physical and functional development. The offspring were studied for survival, fertility and histology. At 15 mg/kg-day male rats had decreased body weight gain. Other reported toxic effects included nephrotoxicity and atrophic gingivitis; however the doses that caused these effects were not reported. Dams in all treatment groups reported were not affected by cyclosporine administration; the only effect was labor dystocia (difficult birth) noted in two high-dose dams. The authors noted that single dams were allowed to litter, and a “relatively high pre-/perinatal mortality” was seen at 15 mg/kg-day, but the effect was not statistically significant. Overall, this study reported minimal paternal toxicity, and no evidence of reproductive or developmental

toxicity at doses up to 15 mg/kg-day.

In an evaluation of perinatal and postnatal toxicity, pregnant female Wistar rats (24/group) were treated with cyclosporine orally (presumably in gelatin capsules) at doses of 5, 15, or 45 mg/kg-day from day 15 postcoitum until 21 postpartum (Ryffel et al., 1983). No toxic effects were observed at 5 and 15 mg/kg-day but a reduction in maternal weight gain was observed at 45 mg/kg-day. Increased offspring mortality (pre-/perinatal and postnatal) and decreased body weight gain were observed at the maternally toxic dose of 45 mg/kg-day.

Male reproductive toxicity has been found in two rat studies (IARC, 1990). In Sprague-Dawley rats administered cyclosporine by gavage at 30 mg/kg-day for four weeks, the levels of serum testosterone were decreased by 50%. In male rats administered cyclosporine sc for 14 days at 10, 20, or 40 mg/kg-day, the body weight, and reproductive tissue weights and histology were examined. The authors reported degenerative changes in the testis, decreases in sperm counts and motility, as well as infertility at the 20 and 40 mg/kg-day doses. In both of these studies the reproductive changes were reported to be reversible after cessation of treatment (IARC, 1990).

4.4.4.5 Genotoxicity

The genotoxicity of cyclosporine has been reviewed in several authoritative assessments (IARC, 1990, 2012; Olshan et al., 1994). Cyclosporine was negative in the *Salmonella tyhimurium* gene mutation assay and for gene mutation at the hprt locus of Chinese hamster V79 cells, both in the presence and absence of an exogenous metabolic system. It was also negative in the dominant lethal assay in CD-1 mice up to 1000 mg/kg.

Cyclosporine did not induce micronuclei in CD mice or Chinese hamsters at doses up to 1500 mg/kg, or chromosome aberrations in human lymphocytes *in vitro*. However, increases in the incidence of chromosomal aberrations have been reported in the lymphocytes of kidney transplant patients, although IARC (1990) noted several limitations in this study. There is also some indication that cyclosporine may cause DNA damage. It induced sister chromatid exchange (SCE) in human peripheral lymphocytes *in vitro*, and unscheduled DNA synthesis (UDS) was increased in the lymphocytes of kidney transplant patients compared to healthy controls. However, UDS was not increased in the germ cells of mice.

Thus, cyclosporine does not cause point mutations, but may cause chromosome damage and other DNA damage. There is general consensus that any genotoxic effects of cyclosporine do not result from direct interaction with DNA. Instead, DNA damage may result from oxidative stress related to cyclosporine exposure.

4.4.4.6 Carcinogenicity

Unlike the clear evidence of carcinogenicity in humans, results from experimental animal studies conducted in mice, rats and monkeys are inconclusive (IARC, 1990; IARC, 2012; NTP, 2014). IARC (2012) concluded that there is “limited evidence in experimental animals” for the carcinogenicity of cyclosporine, but this did not affect the final conclusion that cyclosporine is a Group 1 carcinogen. The following is a brief summary of the animal data:

Ryffel et al. (1983) reported on three studies using oral administration of cyclosporine. Investigations were done in OF 1 mice for 78 weeks, in OFA rats for 104 weeks, and in Beagle dogs for 1 year. In all three studies, the animals were observed daily, and body weights, external masses, and concentrations of cyclosporine in the feed were checked weekly.

- In mice (50 males/50 females per group) cyclosporine was administered in feed at 0, 1, 4, or 16 mg/kg-day for 78 weeks. At the high dose there was an increase in mortality and changes in hematology (slight anemia thrombocytosis). No changes were detected in the frequency, type, or pattern of hyperplastic or neoplastic lesions in mice treated with cyclosporine for 78 weeks at any dose tested.
- In rats (50 males/50 females per group), cyclosporine was administered in feed at 0, 0.5, 2, or 8 mg/kg-day for 2 years. Increased mortality at the high dose was attributed to nephrotoxicity (including an increased extent and severity of strain-specific chronic progressive nephropathy) and hepatotoxicity; pathological evidence of effects on the kidney and liver were also noted at 2 mg/kg-day, but no additional information was provided. Other non-tumor findings at the mid- and high doses included decreased weight gain, anorexia, anemia, and leucopenia. No effects were observed in the 0.5 mg/kg-day group, and there was no evidence of a dose-dependent increase in tumor incidence. Ryffel et al. (1983) concluded that “no carcinogenic potential of cyclosporine could be detected in rats treated for 2 years.”

Three additional animal studies were reported in IARC (1990, 2012); all of these studies were for short terms relative to standard carcinogenicity studies.

- Male AKR mice (30 animals/group) were given cyclosporine, 0 or 150 mg/kg in the diet daily for up to 34 weeks in a screening study. These mice are highly susceptible to the induction of leukemia, and thymic lymphomas were detected beginning at week 17. During weeks 20-29, the incidence of thymic lymphomas in control mice was 2/12 versus treated 13/18. At 30-34 weeks; controls had 3/9 and treated had 9/9 thymic lymphomas. This study reported an increase in lymphoma formation by cyclosporine.
- Male Wistar rats (13-16 rats /group) were treated with streptozotocin (single dose of 60 mg/kg) to induce diabetes, then subsequently treated with cyclosporine by gavage at 0 or 10 mg/kg-day for 20 weeks; it was not clear whether there was a post-dosing observation period. A higher incidence of kidney tumors at $P < 0.05$ was observed in treated animals (7/13) versus controls (2/16).
- In a monkey study, Macaques (n = 55, age and sex not reported) that had received heart or heart lung transplants received daily intramuscular injections of 25 mg/kg-day

cyclosporine for 14 days, and then 17 mg/kg-day every other day or daily. This study also looked at combinations of antithymocyte globulin, azathioprine and methylprednisolone with or without cyclosporine. In monkeys treated with cyclosporine alone, 2/16 monkeys developed B-cell lymphomas, and B-cell lymphomas were seen in 12/55 of all of the monkeys in the study that received cyclosporine alone or in combination with other agents. Interpretation of the results is limited by the absence of control animals, but IARC (1990) noted that lymphomas were not observed in the monkeys receiving only the other immunosuppressive agents, and haematopoietic neoplasms are generally rare in nonhuman primates.

Although increases in tumor incidence were reported in these additional three studies, the models used are nontraditional and have a variety of limitations.

4.4.4.7 Immunotoxicity

As discussed under specific study types, the immunosuppressive effects of cyclosporine are well-characterized and are the basis for its therapeutic use. Histological evidence of effects on immune cells (leukocytosis, lymphopenia, hypochromic anemia, monocytosis and eosinopenia without myelotoxic effects) and atrophied lymphoid tissues was observed in OFA rats fed 45 mg/kg-day or more for 13 weeks (Ryffel et al., 1983). Changes in lymphoid tissue have also been noted in Sprague-Dawley rats fed cyclosporine at 150 ppm in diet for eight weeks, and at 7.5 mg/kg-day or 15 mg/kg-day via oral administration (IARC, 1990).

4.4.4.8 In Vitro Studies and Data Related to Mode of Action

The immunosuppressant mechanism of action for cyclosporine targets T cells, inhibiting signaling pathways originating from binding to intracellular receptors, thereby inhibiting cytokine gene transcription and T cell proliferation. Cyclosporine binds to the cyclophilin receptor and inhibits calcineurin phosphatase activity (Lee, 2010; IARC, 2012). Other cytokines and lymphokines are inhibited by cyclosporine and the overall effect is a reduction in the number and activity of proinflammatory cells at sites of inflammation.

Cyclosporine carcinogenesis is attributed in part to its immunosuppressive activity, resulting in impaired surveillance, particularly for virus-induced cancer. However, comparison with other immunosuppressants indicates that cyclosporine also acts by other mechanisms (IARC, 2012). Another likely component of cyclosporine-induced carcinogenesis is via the induction of tumor-suppressor growth factor (TGF β), a protein synthesized by many tumors and associated with invasiveness. Cyclosporine treatment stimulates TGF β production *in vitro*, and its effects can be blocked both *in vivo* and *in vitro* with TGF β antibodies (IARC, 2012). Cyclosporine has also been reported to alter expression of tumor necrosis factor α (TNF α) in rats (Tedesco and Haragsim, 2012). Both TGF β and TNF α are cytokines that regulate a number of signal transduction pathways involved with inflammatory and acute injuries, chronic inflammation and cancer. Oxidative stress may also play a role in cyclosporine-induced carcinogenicity.

Both nephrotoxicity and hepatotoxicity of cyclosporine are likely to be related to its ability to generate reactive oxygen species (ROS). Cyclosporine has been shown to generate ROS and lipid peroxidation in the rat kidney and liver *in vivo* (Lee, 2010).

Table 11. Summary of Cyclosporine Toxicity Data

Study Type	Route, Species (duration)	References
Toxicokinetics	Extensive human data; 1 oral study in dogs, 3 oral in rats, additional parenteral studies in rats, mice, and rabbits	Ryffel and Mihatsch, 1986 IARC, 1990
Acute lethality	3 oral studies in mice, rats, and rabbits (1 each species); 3 iv studies in mice, rats, and rabbits (1 each species); additional parenteral	Ryffel et al., 1983 IARC, 1990
Acute/short-term toxicity	1 oral study in 5 rat strains for 14 days; 1 gavage study in rats for 21 days; multiple oral studies in rats for an unspecified duration; several parenteral studies in mice, rats and monkeys	Ryffel et al., 1983 IARC, 1990
Repeated dose toxicity	Extensive clinical data in humans; 2 dietary studies in rats for 13 and 78 weeks; 2 oral (gelatin capsule) in monkeys and dogs (13 weeks-1 yr); additional parenteral studies	Ryffel et al., 1983 IARC, 1990
Developmental	1 standard study each in rats and rabbits; 2 additional studies in rats with few details	Ryffel et al., 1983 IARC, 1990
Reproductive	1 standard one-generation oral study in rats and 1 peri-/postnatal study in rats; 2 male reproductive toxicity in rats - 1 sc study for 14 days, 1 gavage for 4 weeks	Ryffel et al., 1983 IARC, 1990
Genotoxicity	1 bacterial gene mutation assay and 1 mammalian cell gene mutation assays; 1 <i>in vitro</i> chromosome aberration; bone marrow micronucleus in mice and hamsters; <i>in vivo</i> UDS and chromosome aberrations in human lymphocytes; germ cell UDS	IARC, 1990, 2012; Olshan et al., 1994
Carcinogenicity	Extensive human clinical data; 1 standard dietary studies in rats (2 years) and mice (78 weeks); short-term studies with nontraditional models in mice and rats; additional parenteral studies not summarized in text; 1 im injection in monkeys with heart-lung allografts	IARC, 1990, 2012 Ryffel et al., 1983

4.4.4.9 Lowest Hazard Endpoints

Cyclosporine is used clinically as an immunosuppressive drug, and therefore, there are extensive data from human exposure. Therapeutic doses are in the range of 2.5 to 12.5 mg/kg-day, for durations ranging from weeks to a year (PDR, 2015). All adverse effects reported in humans occur under the conditions of the therapeutic doses. The most frequent toxic side effect of

cyclosporine is kidney toxicity; of course, the intended effect of therapeutic use (immunosuppression) would also be considered an adverse effect under environmental exposure conditions. Both acute and chronic nephrotoxicity have been reported (Lee, 2010). Other common side effects include hepatotoxicity and nervous system effects. Cyclosporine has a narrow therapeutic index (Da Silva et al., 2014) relative to the induction of nephrotoxicity. The nephrotoxicity observed in humans is supported by several reports of nephrotoxicity in experimental animals. The available short-term studies appear to have evaluated only a limited dose range, but the lowest oral LOAEL was 25 mg/kg-day (based on dosing every other day), based on nephrotoxicity and hepatotoxicity in Sprague-Dawley rats treated by gavage for 21 days (IARC, 1990); no NOAEL was identified. In a 13-week study with OFA rats administered cyclosporine in feed (Ryffel et al., 1983), the LOAEL was 60 mg/kg-day, based on lymphoid atrophy, hepato- and nephrotoxicity, and atrophy of periodontal tissues; the NOAEL was 20 mg/kg-day. The higher effect level (i.e., apparently lower sensitivity) in the 13-week study compared to the 21-day study may reflect strain differences, or the difference between gavage every other day (which results in higher peak blood levels) and daily exposure in feed.

Developmental effects (increased postimplantation loss, decreased weight gain, skeletal retardation) have been observed in rats and rabbits at maternally toxic doses (30 mg/kg-day in rats, and 100 mg/kg-day in rabbits). Postimplantation loss was also increased in rats at 17 mg/kg-day (Ryffel et al., 1983), but the data were presented only on a per pup basis, rather than the more appropriate per litter basis. In a one-generation reproductive toxicity study, no clear evidence of reproductive toxicity was seen at doses up to 14 mg/kg-day (Ryffel et al., 1983). There is some evidence of reversible male reproductive toxicity. Decreased testosterone was seen in rats administered 30 mg/kg-day for 14 days, and lower sperm counts and sperm motility (IARC, 1990). However, there was no effect on male fertility in the reproductive toxicity study conducted that resulted in decreased body weight gain in males, and nephrotoxicity in some animals (Ryffel et al., 1983).

Cyclosporine is classified as a known human carcinogen (NTP, 2014) with an incidence of ~5% in patient populations (IARC, 1990). Data in experimental animals are much weaker (IARC, 2012). Cyclosporine does not cause gene mutations, but there is some evidence that it is clastogenic, and it may cause DNA damage (IARC, 1990, 2012; Olshan et al., 1994). The mode of action for any DNA damage is likely oxidative stress, rather than direct interaction with DNA. The cancer mode of action is likely immunosuppression and perhaps also induction of TGF β and oxidative stress (IARC, 2012).

Persons with kidney disease are more susceptible to the adverse renal effects and apparently the immunosuppressive effects of cyclosporine.

Table 12. Lowest Hazard Endpoints for Cyclosporine

Species/ Route	Duration	NOAEL/ LOAEL	Effect/Comments	Citation
Humans/ oral	Weeks- 1 year	LOAEL 2.5 mg/kg-day	Immunosuppression Most common side effect: kidney toxicity; also hepatotoxicity, nervous system effects, and cancer. Rich database.	PDR, 2015
Sprague Dawley rats/gavage	21 days	LOAEL 25 mg/kg-day	Nephrotoxicity; hepatotoxicity	IARC, 1990
OFA rats/feed	13 weeks	NOAEL 20 mg/kg-day; LOAEL 60 mg/kg-day	Lymphoid atrophy, hepato- and nephrotoxicity, and atrophy of periodontal tissues	Ryffel et al., 1983
OFA rats/ gelatin (capsule?)	Develop- mental: Postcoital days 6-15	NOAEL (?) 17 mg/kg- day; Maternal and Developmental LOAEL 30 mg/kg-day	Decreased maternal weight gain and increased postimplantation loss, decreased weight gain, skeletal retardation). Postimplantation loss increased at 17 mg/kg-day, but data were presented only on a per pup basis,	Ryffel et al., 1983
Wistar rats/ gelatin (capsule?)	Reproductive : Males from 12 weeks pre-mating, females through weaning	NOAEL 5 mg/kg-day in males; LOAEL 15 mg/kg-day Reproductive NOAEL 15 mg/kg-day	Males had decreased body weight gain. No clear reproductive effects, although 2 females at 15 mg/kg-day had difficult labor	Ryffel et al., 1983

4.4.5 References

da Silva, J. B., de Melo Lima, M. H., Secoli, S. R., 2014. Influence of cyclosporine on the occurrence of nephrotoxicity after allogeneic hematopoietic stem cell transplantation: A systematic review. *Rev Bras Hematol Hemoter.* 6, 363–368.

Gratwohl, A., Riederer, I., Graf, E., Speck, B., 1986. Cyclosporine toxicity in rabbits. *Lab Anim.* 20, 213-220.

HSDB (Hazardous Substances Data Bank). 2009. Cyclosporin A. National Library of Medicine. Available at: <http://toxnet.nlm.nih.gov/cgi-bin/sis/search/a?dbs+hsdb:@term+@DOCNO+6881>.

IARC (International Agency for Research on Cancer). 2012. Cyclosporin. IARC Monographs 100A. International Agency for Research on Cancer. p. 337-345.

IARC (International Agency for Research on Cancer). 1990. Cyclosporin. IARC Monographs 50. International Agency for Research on Cancer. p. 77-114

Lee, J., 2010. Use of antioxidants to prevent cyclosporine toxicity. *Toxicol. Res.* 26, 163-170.

NTP (National Toxicology Program). 2014. Cyclosporin A, *Report on Carcinogens, Thirteenth Edition*. U.S. Department of Health and Human Services, Public Health Service, National Toxicology Program. Research Triangle Park, NC.

Olshan, A. F., Mattison, D. R., Zwanenburg, T. S., 1994. International Commission for Protection Against Environmental Mutagens and Carcinogens. Cyclosporine A: review of genotoxicity and potential for adverse human reproductive and developmental effects. *Mutat Res.* 317, 163-173.

PDR (Physicians' Desk Reference). 2015. Drug information - Cyclosporine. Available at: <http://www.pdr.net/search-results?q=cyclosporine>

Ragab, A. R., Al-Mazroua, M. K., Al-Dakrory, S. A., 2013. Cyclosporine toxicity and toxicokinetics profiles in renal transplant recipients. *J Clinic Toxicol.* 3, 154-162.

Ryffel, B., Donatsch, P., Madörin, M., Matter, B. E., Rüttimann, G., Schön, H., Stoll, R., Wilson, J., 1983. Toxicological evaluation of cyclosporin A. *Ach Toxicol.* 53, 107-141.

Ryffel, B., Mihatsch, M. J., 1986. Cyclosporine nephrotoxicity. *Toxicol Pathol.* 14, 73-82.

Tedesco, D., Haragsim, L., 2012. Cyclosporine: A review. *J Transplant.* 2012, 1-7.

4.5 Citrinin

4.5.1 Physical and Chemical Characteristics

Table 13. Physical and Chemical Characteristics of Citrinin

Characteristic		Reference
Molecular formula	C ₁₃ H ₁₄ O ₅	EFSA, 2012
IUPAC	(3R, 4S)-4,6-dihydro-8-hydroxy-3,4,5-trimethyl-6-oxo-3H-2-benzopyran-7-carboxylic acid	EFSA, 2012
Cas No	518-75-2	EFSA, 2012
Molecular weight	250.25	EFSA, 2012

Characteristic		Reference
Melting Point	175 °C	EFSA, 2012
Physical State	Acidic lemon-yellow crystalline solid	IARC, 1986; EFSA, 2012
Solubility	Practically insoluble in water; soluble in aqueous sodium hydroxide, sodium carbonate, methanol, ethanol, most polar organic solvents	IARC, 1986; EFSA, 2012
Decomposition	Occurs at > 175 °C under dry conditions, and at > 100 °C in the presence of water; decomposes to citrinin H1 and citrinin H2	EFSA, 2012

4.5.2 Toxicokinetics

Very limited data were located on the toxicokinetics of citrinin, and the few data that are available were based on either injection exposure or nonstandard animal models. No standard toxicokinetic studies were available.

No data were located on the absorption of citrinin via the oral or inhalation routes. The observation of systemic toxicity following oral exposure and excretion via the urinary route indicates that oral absorption occurs, but the data are insufficient to estimate the rate or extent of absorption. In an *in vitro* human skin model, citrinin was shown to penetrate through the skin (Boonen et al., 2012).

An iv injection of 3 mg/kg in rats was rapidly distributed to the liver and kidneys (IARC, 1986), consistent with the observed targets for toxicity. Plasma levels in pigs gavaged with 0.02 mg/kg-day daily for 8 weeks were slightly higher at week 8 compared to week 6, indicating that there is not substantial accumulation in the tissues that equilibrate with plasma at this dose in pigs. IARC (1986) reported that citrinin crossed the placenta in rats after sc injection on GD 12.

No data were located on citrinin metabolism.

Pregnant rats rapidly excreted ¹⁴C-citrinin in urine (~75%) following sc injection of 35 mg/kg on GD 12, with almost 75% of the dose being eliminated in urine within the first 24 hours (IARC, 1986). The elimination from plasma was biphasic, with half-lives of about 2 and 40 hours. An iv injection of 3 mg/kg in rats was rapidly distributed to the liver and kidneys and excreted primarily in the urine, and to a lesser extent in the feces (IARC, 1986).

4.5.3 Hazard Information - Human

No epidemiology studies were reported that evaluated the potential association of citrinin with any effects (IARC, 1986; EFSA, 2012).

Very limited data suggest that citrinin is a nasal irritant. EFSA (2012) summarized a report from the 1930's that human inhalation of an unspecified exposure to micro-crystalline citrinin produced "violent fits of sneezing". Similarly, a report from the 1940's indicated that inhalation of citrinin dust resulted in mucosal irritation (EFSA, 2012).

EFSA (2012) reported that the occurrence of citrinin in grains has been linked to so-called Balkan endemic nephropathy (BEN). However, the cited studies only reported the presence and amount of citrinin in the endemic areas and did not conduct any further analysis to test the apparent association.

4.5.4 Hazard Information - Animal

4.5.4.1 *Acute Lethality*

EFSA (2012) reported LD₅₀ studies in several species by oral and parenteral routes. Oral LD₅₀s in mice and rabbits were 105-134 mg/kg. LD₅₀ values in rats, mice, rabbits and guinea pigs exposed by sc or ip injection were 35-89 mg/kg. The lowest reported LD₅₀ was 19 mg/kg in rabbits exposed iv. This difference may reflect the toxicokinetics following iv injection, rather than species differences, in light of the similarity of oral LD₅₀s in rats and rabbits. Dyspnea, lacrimation and histopathological changes in the spleen and kidney were common findings in these studies.

4.5.4.2 *Acute/Short-term Toxicity*

No data regarding primary skin irritation or sensitization were reported in EFSA (2012).

One study in rabbits showed conjunctival irritation when 2% aqueous liquid of citrinin was applied to the eye (EFSA, 2012).

Single ip doses of 35-50 mg/kg produced liver and kidney pathological changes, transient renal damage and nephrosis in mice and rats (EFSA, 2012). Mice showed a mild decrease in immune response when injected (route not specified) with a single dose of 2.5 mg/kg (EFSA, 2012).

EFSA (2012) cites 3 studies in guinea pigs and dogs treated via ip injection with 50 mg/kg daily for 2-14 days, 5-10 mg/kg for 2 weeks or 20-40 mg/kg for 5 days. These studies reported congested, swollen or necrotic kidneys, clinical signs of kidney disease or lethality (10 mg/kg for 7-11 days in dogs).

EFSA (2012) reported that intravenous doses of 5-20 mg in dogs decreased blood pressure.

In two dietary studies in hamsters and rats, citrinin was administered for 5-14 days at doses of 18-50 mg/kg with no adverse effects reported (EFSA, 2012). An oral study in guinea pigs for 14 days produced lethality at 43 mg/kg/day (mean lethal dose) (EFSA, 2012). Mice showed

multiple immune modulatory effects (e.g., changes in spleen cell populations and serum immunoglobulin) upon administration of 1-10 mg/kg orally for 2 weeks (Islam et al., 2012). The LOAEL for immune modulatory effects is 1 mg/kg-day.

4.5.4.3 Repeated Dose Toxicity

No adverse effects were reported in pigs gavaged with 0.02 mg/kg-day for up to 57 days (Sandor et al., 1991). Endpoints evaluated included body weight, weights of major organs, histopathology of major organs, hematology and clinical chemistry. The NOAEL for this study is 0.02 mg/kg-day. Similarly, no adverse effects were reported when Lee et al. (2010) fed male Wistar rats 0, 2 ppm or 200 ppm citrinin in rice administered at 100 mg/kg body weight/day for 90 days. EFSA (2012) calculated that these concentrations corresponded to 0, 0.2 or 20 µg/kg-day (2E-4 to 0.02 mg/kg-day). Renal effects were also seen in dogs fed diets that included rice containing *Penicillium citrinum* mold (EFSA, 2012). The most sensitive endpoint was a marked increase in lactate dehydrogenase in urine of dogs dosed orally with 5 mg/kg-day for an unspecified period at levels that did not result in clinical signs of kidney toxicity.

When mice received 0.12-3.0 mg/kg ip every other day for 2 or 4 weeks, mild immune stimulation was reported (EFSA, 2012). At 20 mg/kg ip once per week for 6 weeks, decreased total bone marrow cell count was reported in mice (EFSA, 2012).

IARC (1986) cited repeat-dose studies in rabbits, pigs and rats. Nephrotoxic effects were reported in rabbits receiving 20 mg/kg-day iv for 8 weeks, pigs dosed orally for 70 days with 20 mg/kg-day and rats dosed orally with 14 mg/kg-day for 15 days.

These studies indicate that key targets of citrinin toxicity are the kidney and potentially the immune system. Additional data from repeated dose studies are summarized in the context of the carcinogenicity data.

4.5.4.4 Developmental and Reproductive Toxicity

Singh et al. (2014) fed male and female Wistar rats (25/group) 0, 1, 3 or 5 ppm citrinin (in the form of cultured maize powder containing known amounts of citrinin) in diet (approximately 0, 0.01, 0.03 or 0.05 mg/kg-day, based on the food factor for a subchronic study in Wistar rats of 0.1 (U.S. EPA, 1988) for 10 weeks prior to, and during, mating. Ten pregnant females from each of these groups were fed 0, 1, 3 or 5 ppm citrinin in the diet until day 20 of pregnancy. Clinical observations and body weights were assessed until sacrifice on GD 20. Maternal endpoints included: number of corpora lutea, number of implants, resorptions and live and dead fetuses. Skeletal and visceral examinations were performed on the fetuses. Dams in all treatment groups showed clinical signs prior to mating, such as increased water intake and polyuria, and rough hair coat; there was no mortality; clinical signs (further details not reported) were also seen in the

pregnant animals. While maternal body weight for the all treatment groups was significantly different from control at day 0 (indicating pre-pregnancy toxicity from the exposure 10 weeks prior to mating) and throughout gestation, the percentage increase in body weight gain during pregnancy was similar. At 3 and 5 ppm, the number of resorptions, resorptions as percent of implants, and post implantation loss were increased, whereas fetal weight and crown-rump length were decreased relative to controls. At 5 ppm, the percent of live fetuses was decreased, and gross anomalies, and skeletal and visceral malformations were increased (enlarged renal pelvis, hydrocephaly, microphthalmia, incomplete ossification of skull bones) when compared with controls. Based on the decreased body weight and clinical signs consistent with kidney toxicity in the female rats at all doses prior to mating, 1 ppm is a LOAEL in the dams. The LOAEL for developmental toxicity was 3 ppm based on increased resorptions, post implantation loss, and decreased fetal weight and length, and the developmental NOAEL for this study is 1 ppm. Since the females were exhibiting adverse effects at all doses prior to getting pregnant, at a dose lower than the developmental NOAEL in this study, the developmental effects may be secondary to maternal toxicity.

Four developmental studies have been reported by IARC (1986) and EFSA (2012). In the only oral study, citrinin was administered in the diet to pregnant Wistar rats at 10 ppm (reported by EFSA, 2012 to be about 1 mg/kg-day) on GD 6-20. Mild to moderate maternal toxicity (degenerative liver changes, renal lesions, reduced weight gain and feed intake, polydypsia and polyuria) was reported. Embryo/fetal effects included retarded growth, increased fetal resorption rate, and severe malformations (hydrocephalus, cleft palate), in addition to evidence of systemic toxicity (enlarged kidneys, renal tubular necrosis and degeneration). EFSA also noted that deviation from the target dose in this study was considerable and contributed to the study uncertainty.

Two studies wherein citrinin was administered via single sc injection in pregnant rats during gestation reported maternal and fetal effects (IARC, 1986). Dams were given a single dose of 35 mg/kg one day during the gestation period of days 3-15 (GD 3-15) in one study. In the other study, a single dose of 30 mg/kg was given during GD 5-11 or on GD 14. Compromised body weight gain was seen in one study and maternal lethality in both studies. Live fetuses and fetal weight were reduced and minor fetal anomalies occurred in one of the studies. In mice administered 10-40 mg/kg ip on one of GD 6-9, decreased fetal weight gain and increased fetal lethality was reported at 30 and 40 mg/kg, but “occasional” maternal lethality was also reported (IARC, 1986). No fetal abnormalities were reported in the latter study.

No standard reproductive toxicity studies were identified. An oral one-generation study in rats investigated only oxidative stress and apoptosis in the liver, kidney and testes, not functional or histopathology indicators of reproductive endpoints (Singh et al., 2013). Apoptosis and

oxidative stress was noted in the liver, kidney and testes of both generations. Intraperitoneal injections of male mice with 0.0625-6.25 mg/kg daily for 7 days increased weight and histological alteration of reproductive and accessory organs, decreased sperm count, increased abnormal sperm, decreased testosterone and decreased pregnancy rate; there were no embryos when the high-dose males were mated with untreated females (Qingqing et al., 2012; EFSA, 2012).

Overall, a number of studies in mice and rats using both oral and parenteral dosing reported developmental toxicity, including malformations. However, the data are insufficient to determine whether citrinin is a direct developmental toxicant, since all developmental toxicity either occurred above the maternal LOAEL, or occurred in the presence of severe maternal toxicity (lethality), when only one dose was tested. The mechanistic evidence (oxidative stress) and results from parenteral dosing also indicate that citrinin causes male reproductive toxicity, but information is lacking on standard endpoints following exposure via an environmentally relevant route.

4.5.4.5 Genotoxicity

The weight of evidence for gene toxicity indicates that citrinin is negative for gene mutation in bacteria; there are no gene mutation assays in mammalian cells or *in vivo* (IARC, 1986; EFSA, 2012). Citrinin causes chromosome damage *in vivo* (micronuclei) and *in vitro* in a variety of animal cell models and in human lymphocytes. Several other studies (e.g., sister chromatid exchange assay) have shown primarily negative results for DNA damage.

4.5.4.6 Carcinogenicity

Arai and Hibino (1983) fed F344 male rats (22 controls, 50 exposed) with 0 or 0.1% (1000 ppm in the diet, estimated by EFSA [2012] as corresponding to 70 mg/kg-day) citrinin *ad libitum* in the diet for up to 80 weeks. Animals were weighed weekly and control (5-10) and experimental animals (8-17) were sacrificed at weeks 32, 40, 60 and 80 weeks. Kidney, liver, lung and spleen were examined histopathologically; kidneys were also examined ultrastructurally. Throughout the experiment, citrinin-fed animals showed decreased body weight relative to controls. At each time point, mean kidney weight and relative kidney weight were increased in the treatment group. Relative liver weight was increased at week 80 in treated animals. At week 32, the kidneys of treated animals (13/13) showed focal hyperplasia, marked proximal tubular dilatation, and interstitial fibrosis. At week 40, focal hyperplasia and small adenomas were noted in the kidney. Large renal clear cell adenomas were found after 60 weeks. No tubular dilation, focal hyperplasia or tumors were found in controls. Tubular dilation and focal hyperplasia were reported in all treated animals. Renal adenoma incidence in treated rats at 32, 40, 60, 80 weeks were as follows: 0/13, 8/8, 17/17, 10/10. Although carcinomas were not reported, EFSA (2012) noted that carcinomas may have formed if the study were carried out for the standard duration of

carcinogenicity studies. Because only one dose was tested, no dose-response analysis is possible from this study, but the single dose tested (70 mg/kg-day) was a LOAEL for both kidney toxicity and an effect level for tumors.

IARC (1986) reported two additional oral carcinogenicity studies in mice and rats. Mice (20/group) fed diets of 0, 100 or 200 ppm citrinin for 70 weeks showed no differences in survival and did not develop renal tumors. Rats (groups of 10-20 male Sprague Dawley) fed diets containing 0, 0.02 or 0.05% (200 or 500 ppm, about 25 or 70 mg/kg-day) citrinin for 48 weeks showed no kidney tumors. Both studies are limited by the short duration and small sample sizes.

When NDMA was administered to groups of 20 Sprague Dawley rats as an initiator followed by citrinin at 0.02 or 0.05% (~25 or 70 mg/kg-day) in the diet for 20 weeks, significant increases in renal cell adenomas were produced in treated rats (18/19, 13/15) relative to positive controls receiving NDMA alone (2/14) (IARC, 1986; EFSA, 2012). A higher incidence of renal cell tumors was reported in male DDD mice (groups of 20) that received diets containing 25 ppm ochratoxin A and 200 ppm citrinin compared with mice receiving ochratoxin A alone (10/18 vs. 6/18) (IARC, 1986). The study is limited by the small sample size and no statistical analysis was provided by IARC (1986).

In summary, IARC (1986) concluded that there was “limited evidence for the carcinogenicity of citrinin to experimental animals” and that “no evaluation could be made of the carcinogenicity of citrinin to humans.” Citrinin is classified in group 3 (not classifiable as to its carcinogenicity to humans). Although this assessment is almost 30 years old, no additional data are available to modify this conclusion.

4.5.4.7 *In Vitro Studies and Data Related to Mode of Action*

A number of *in vitro* studies have been conducted to investigate the mode of action for effects on the kidney, as well as to further investigate potential developmental/reproductive effects. Several *in vitro* studies with citrinin have reported increased apoptosis in mouse and rat test systems (EFSA, 2012; Liu et al., 2012). Decreased implantation rates, and reduction in oocyte maturation rate, fertilization and embryonic development were found in mouse blastocysts and embryoblasts showing increased apoptosis. Similarly, Liu et al. (2012) found induction of apoptosis decreased testosterone production in rat Leydig cells. In a review, Doi and Uetsuka (2014) summarized *in vitro* and *in vivo* reports indicating that citrinin causes oxidative stress that leads to apoptosis, most notably in mouse skin.

EFSA (2012) reported decreased production of interferon, without cytotoxicity, in an assay for cell viability and proliferation in human peripheral blood mononuclear cells.

Overall, EFSA (2012) concluded that citrinin causes oxidative stress, perhaps as a result of alteration of mitochondrial functionality, and resulting in apoptosis. Inhibition of microtubule assembly was also noted.

Table 14. Summary of Toxicity Data for Citrinin

Study Type	Route, Duration	References
Toxicokinetics	1 sc in pregnant rats, 1 iv study in rats, 1 oral study in pigs, 1 <i>in vitro</i> study with human skin	Boonen et al., 2012; Sandor et al., 1991; EFSA, 2012
Acute lethality	3 oral studies in mice and rabbits (LD ₅₀ =105-134 mg/kg); 4 sc and 8 ip studies in rats, mice, guinea pigs and rabbits (LD ₅₀ = 35-89 mg/kg); 1 iv study in rabbits (LD ₅₀ =19 mg/kg)	EFSA, 2012
Acute/short-term toxicity	6 ip studies in guinea pig, dog for 2-14 days; 2 dietary studies in hamsters and rats for 5-14 days ; 2 oral studies in guinea pig and mice for 5-14 days	Islam et al, 2012; EFSA, 2012
Primary Irritation	1 eye irritation study in rabbits No data for skin irritation	EFSA, 2012
Sensitization	No data	
Repeated dose toxicity	2 ip studies in mice for 2-6 weeks; 1 iv study in rabbits for 8 weeks; 3 oral studies- 2 in pigs for 57-70 days and 1 in rats for 15 days; 2 dietary studies-1 in dogs for 32 days and 1 in rats for 90 days	IARC, 1986; EFSA, 2012
Developmental	3 studies in rats- 1 dietary, 2 subcutaneous: 1 dietary and 1 ip study in mice	Singh et al., 2014; IARC, 1986; EFSA, 2012
Reproductive	1 nonstandard one-generation dietary study in rats; 1 ip male reproductive toxicity study in mice	Singh et al. 2013; Qingqing et al., 2012
Genotoxicity	6 bacterial gene mutation assays; 3 mammalian cell gene mutation assays; 2 <i>in vitro</i> and 1 <i>in vivo</i> assay of chromosome damage; 3 other assays evaluating DNA damage	IARC, 1986; EFSA, 2012
Carcinogenicity	3 dietary studies, 1 in mice for 70 weeks and 2 in rats for 48 and 32-80 weeks; 1 initiation-promotion carcinogenicity study	IARC, 1986; EFSA, 2012; Arai and Hibino, 1983

4.5.5 Lowest Hazard Endpoints

The most prominent adverse effect of citrinin is on the kidney. Studies in multiple mammalian species by oral, dietary, intravenous, subcutaneous and intraperitoneal routes for durations ranging from acute to chronic have shown renal tubular dilatation, nephrosis, necrosis and (in one case) tumors. Limited study designs preclude deriving definitive NOAEL/LOAELs for systemic toxicity. A NOAEL of 0.02 mg/kg-day with no corresponding LOAEL was identified in pigs administered citrinin for up to 57 days (Sandor et al., 1991) and in rats fed citrinin for 90 days (Lee et al., 2010). However, there is considerable uncertainty in identifying the NOAEL-LOAEL boundary. In the repeat-dose studies at the next-highest doses, pigs dosed orally with 20 mg/kg-day for 70 days showed nephrotoxic effects (IARC, 1986) and rats administered an estimated dose of 70 mg/kg-day for up to 80 weeks had increased liver weight and kidney histopathology (hyperplasia, tubular dilatation, fibrosis) (Arai and Hibino, 1983). These LOAELs are several orders of magnitude above the NOAELs. EFSA (2012) noted that there is considerable uncertainty regarding the effect level for nephrotoxicity, but used the NOAEL of 0.02 mg/kg-day to identify a dose that would not be of concern for nephrotoxicity in humans.

Adverse effects have also been reported in the liver at high doses, but no liver effects were reported in the key repeated dose studies (Arai and Hibino, 1983; Lee et al., 2010). It is clear that the kidney is more sensitive than the liver.

Several studies have reported effects on the immune system, but the data are inconsistent regarding the nature and direction (e.g., immune stimulation vs. immunotoxicity) of the effect. EFSA (2012) concluded that “the studies of the immunotoxicity of citrinin are incomplete and often non-specific and do not allow a conclusive evaluation.”

A number of studies in mice and rats using both oral and parenteral dosing reported developmental toxicity, including malformations. However, the data are insufficient to determine whether citrinin is a direct developmental toxicant, since all developmental toxicity either occurred above doses that caused effects in the dams, or occurred in the presence of severe maternal toxicity (lethality). Furthermore, limitations in the study (e.g., deviation from the target dose) limit the reliability of identified effect levels. In the single available oral developmental toxicity study that tested multiple dose levels, the LOAEL in the female rats was 1 ppm in diet (about 0.01 mg/kg-day) (based on effects seen prior to mating), and the developmental LOAEL was 3 ppm in diet (about 0.03 mg/kg-day), based on increased resorptions, post implantation loss, and decreased fetal weight and length (Singh et al., 2014). It is noted that the maternal LOAEL is lower than the NOAEL identified in other studies in the same strain of rats (Lee et al., 2010), suggesting that female rats are more sensitive than males, or that there may be issues with the Singh et al. (2014) study. No standard studies were located that investigated reproductive effects or histopathology of the reproductive organs following dosing via an environmentally relevant route. However, male reproductive effects (e.g., histopathology, decreased sperm count,

decreased testosterone) were noted in mice given ip injections of citrinin for 7 days (EFSA, 2012).

Citrinin induced kidney tumors as early as 40 weeks in rats exposed to 1000 ppm citrinin in the diet (approximately 70 mg/kg-day) (Arai and Hibino, 1983). Kidney tumors were also seen in an initiation-promotion study in rats (IARC, 1986; EFSA, 2012). No tumors were found in two oral carcinogenicity studies in mice fed citrinin in diet at 200 ppm for 70 weeks or in rats fed up to 500 ppm in diet for 48 weeks. However, these negative studies tested lower doses than tested in the positive carcinogenicity study, were of short duration, and had small sample sizes. Overall IARC (1986) concluded that there was “limited evidence for the carcinogenicity of citrinin to experimental animals” and that “no evaluation could be made of the carcinogenicity of citrinin to humans.” Citrinin is classified in group 3 (not classifiable as to its carcinogenicity to humans).

Limited data indicate that citrinin is an eye irritant and may be a respiratory irritant.

There is uncertainty in the database as to the dose-response for kidney effects and the potential for immune effects. Rigorous subchronic and chronic studies by nonparenteral routes are needed.

Persons with kidney disease may be more susceptible to the adverse renal effects of citrinin.

Table 15. Lowest Hazard Endpoints for Citrinin

Species/ Route	Duration	NOAEL/ LOAEL	Effect/Comments	Citation
Pigs/ gavage	57 days	NOAEL 0.02 mg/kg-day; No LOAEL	No adverse effects. Low confidence in NOAEL/LOAEL bound	Sandor et al., 1991
Rat/feed	90 days	NOAEL 0.02 mg/kg-day; No LOAEL	No adverse effects	Lee et al., 2010
Wistar rat/feed	10 weeks prior to, during mating, and through GD 20	Maternal NOAEL – none, LOAEL - 1 ppm (~0.01 mg/kg-day); Developmental NOAEL- 1 ppm (~0.01 mg/kg-day), LOAEL 3 ppm (~0.03 mg/kg-day)	Maternal: Decreased body weight Developmental: Increased resorptions, post implantation loss; decreased fetal weight	Singh et al., 2014
			Not classifiable as to its carcinogenicity in humans	

4.5.6 References

- Anninou, N., Chatzaki, E., Papachristou, F., Pitiakoudis, M., Simopoulos, C. 2014. Mycotoxins' activity at toxic and sub-toxic concentrations: differential cytotoxic and genotoxic effects of single and combined administration of sterigmatocystin, ochratoxin A and citrinin on the hepatocellular cancer cell line Hep3B. *Int. J. Environ. Res. Public Health* 11(2), 1855-1872.
- Arai, M., Hibino, T. 1983. Tumorigenicity of citrinin in male F344 rats. *Cancer Letters* 17, 281-287.
- Boonen, J., Malysheva, S.V., Taevernier, L., Diana Di Mavungu, J., De Saeger, S., De Spiegeleer, B. 2012. Human skin penetration of selected model mycotoxins. *Toxicology* 301(1-3), 21-32.
- Doi, K., Uetsuka, K. 2014. Mechanisms of mycotoxin-induced dermal toxicity and tumorigenesis through oxidative stress-related pathways. *J.Toxicol.Pathol.* 27, 1-10.
- EFSA (European Food Safety Authority). 2012. EFSA Panel on Contaminants in the Food Chain (CONTAM); Scientific Opinion on the risks for public and animal health related to the presence of citrinin in food and feed. *EFSA Journal* 2012 10(3), 2605.
- IARC (International Agency for Research on Cancer), 1986. Citrinin. Some Naturally Occurring and Synthetic Food Components, Furocoumarins and Ultraviolet Radiation. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, vol. 40. International Agency for Research on Cancer.
- Islam, M.R., Roh, Y.S., Cho, A., Kim, J., Kim, J.H., Eo, S.K., Lim, C.W., Kim, B. 2012. Immune modulatory effects of the foodborne contaminant citrinin in mice. *Food Chem. Toxicol.* 50(10), 3537-3547.
- Lee, C. H., Lee, C.L., Pan, T.M. 2010. A 90-d toxicity study of monascus-fermented products including high citrinin level. *J. Food Sci.* 75, T91-97.
- Liu, S., Wang, D., Zhang, J., Zhang, D., Gong, M., Wang, C., Wei, N., Liu, W., Wang, Y., Zhao, C., Cui, Y., Hu, D. 2012. Citrinin reduces testosterone secretion by inducing apoptosis in rat Leydig cells. *Toxicol. In Vitro* 26(6), 856-861.
- Qingqing, H., Linbo, Y., Yunqian, G., Shuqiang, L. 2012. Toxic effects of citrinin on the male reproductive system in mice. *Exp. Toxicol. Pathol.* 64(5), 465-469.
- Sándor, G., Busch, A., Watzke, H., Reek, J., Ványi, A. 1991. Subacute toxicity testing of ochratoxin-A and citrinin in swine. *Acta Veterinaria Hungarica* 39, 149-160.

Singh, N.D., Sharma, A.K., Dwivedi, P., Leishangthem, G.D., Rahman, S., Reddy, J., Kumar, M. 2013. Effect on feeding graded doses of citrinin on apoptosis and oxidative stress in male Wistar rats till F1 generation. *Toxicol. Ind. Health*. 2013 Oct 8. [Epub ahead of print](Abstract)

Singh, N.D., Sharma, A.K., Patil, R.D., Rahman, S., Leishangthem, G.D., Kumar, M. 2014. Effect of feeding graded doses of Citrinin on clinical and teratology in female Wistar rats. *Indian J. Exp. Biol.* 2(2), 159-67.

U.S. EPA (U.S. Environmental Protection Agency) 1988. Recommendations for and documentation of biological values for use in risk assessment. EPA/600/6-87/008. Office of Research and Development. U.S. Environmental Protection Agency.

4.6 Ochratoxin

4.6.1 Physical and Chemical Characteristics

There are two types of ochratoxins, ochratoxin A (OTA) and ochratoxin B (OTB). OTA is the most prevalent and toxic; OTB appears to be relevant primarily as a low-level contaminant in some of the naturally contaminated material tested in some animal toxicity studies (EFSA, 2006). Minimal data were located on the toxicity of OTB alone, and so this document focuses on OTA, because it is a ubiquitous and highly toxic mycotoxin.

Table 16. Physical and Chemical Characteristics of Ochratoxin

Characteristic ¹		Reference
Molecular formula	C ₂₀ H ₁₈ ClNO ₆	NTP, 1989
IUPAC	(R)-N[(5-chloro-3,4-dihydro-8-hydroxy-3-methyl-1-oxo-1H-2-benzopyran-7-yl)-carbonyl](-L-)phenylalanine (OTA) N-[(8-hydroxy-3-methyl-1-oxo-3,4-dihydro-1H-isochromen-7-yl)carbonyl]-(R)-L-phenylalanine (OTAB)	EFSA, 2006
CAS No	CAS No. 303-47-9C (OTA) CAS No. 4825-86-9 (ochratoxin B)	EFSA, 2006
Molecular weight	403.8	NTP, 1989
Melting Point	169°C	NTP, 2014
Physical State	White crystalline powder	NTP, 1989

Characteristic ¹		Reference
Solubility	The free acid is insoluble in water but is moderately soluble in organic solvents such as chloroform, ethanol, methanol, and xylene	NTP, 2014
Decomposition	Unstable in light, Fairly stable in heat	NTP, 2014

¹OTA unless otherwise stated.

4.6.2 Toxicokinetics

Data from several animal species show that OTA is rapidly absorbed after oral ingestion and reaches the systemic circulation (EFSA, 2006; Vettorazzi et al., 2014). Once in the blood stream, ~99% of the OTA becomes bound to serum proteins. This binding is one of the reasons for the long half-life of OTA (varying from 4.1 to 510 hours, depending on the species and the route of exposure, with the longest half-life following iv administration) (EFSA, 2006). The binding capacity varies with the species, with human > rat > pig > mouse. This order of relative capacity correlates with the biological half-life (Pfohl- Leszkowicz and Manderville, 2007). OTA and its metabolites are primarily found in the kidney, consistent with the kidney being the main target organ for OTA toxicity. In contrast, OTB was extensively metabolized and not retained in the kidney after treatment; EFSA (2006) suggested that this difference in toxicokinetics is why OTB is less toxic. OTA is also found in liver, lung, and heart. OTA crosses the placenta and has also been detected in the milk (JECFA, 2001; EFSA, 2006).

The metabolism of OTA has been extensively studied, but the details are unclear and controversial. OTA is detoxified mainly by hydrolysis to a metabolite that is less toxic than the parent compound. In rats, approximately 25-27% of an ip or oral dose is present in the urine as the hydrolysis metabolite (JECFA, 2001). In addition, OTA is metabolized to 4-hydroxyl-OTA and other oxygenated metabolites by CYP-450 enzymes located in the liver. The oxidative metabolism is hypothesized to be followed by glucuronide conjugation (EFSA, 2006). The contribution of oxidative metabolism to detoxification of OTA varies with the species and strain tested. Since the various metabolites of OTA vary in toxicity, this has complicated the evaluation of the toxicity of OTA (Pfohl-Leszkowicz and Manderville, 2007; Vettorazzi, 2014). Another source of interspecies and interindividual variability is differences in the entero-hepatic circulation of OTA-glucuronides (EFSA, 2006).

In humans, the plasma half-life of OTA from one human volunteer who ingested 395 ng of radiolabelled OTA was 35 days when calculated using a two-compartment model⁴ (EFSA,

⁴EFSA (2006) stated that recent data on accumulation in the kidney suggest that multi-compartment models are more appropriate, but it was inconsistent regarding the appropriateness of a two-compartment model and it did not provide a reference for the initial statement about recent data.

2006). When the OTA plasma concentrations (apparently resulting from incidental consumption in food) were measured in 8 volunteers from Italy over a 2 month period, renal clearance was calculated to be 0.13 L/day. In vervet monkeys treated with 0.8-2 mg/kg iv, clearance from plasma appeared to follow a two-compartment model, with a half-life of 19-21 days, average clearance of 0.22 mL/hr-kg, and an apparent mean volume of distribution of 59 mL/kg for both the central and peripheral compartments (EFSA, 2006). The major analyte in human serum is OTA and only a small percentage of metabolites have been detected. In the urine, 50% of the material from OTA exposure appears as the parent compound (OTA), suggesting that 50% is metabolites or OTA –glucuronide conjugates (EFSA, 2006). The excretion half-life of OTA in rodents ranges from 53-258 hrs (approximately 2¼ - 11 days) (Vettorazzi et al., 2014) and is 5-6 days in pigs. In humans, the elimination half-life is estimated at 35 days based on data from one individual (EFSA, 2006).

Both biliary and renal routes are involved in the excretion of OTA in rats and mice, with the ratio of the routes dependent on the dose and the route of administration. In humans and nonhuman primates, OTA is excreted mainly via the kidney, whereas biliary excretion predominates in rodents (EFSA, 2006; Vettorazzi et al., 2014).

4.6.3 Hazard Information- Human

OTA is a widespread contaminant of food and animal feed and likely results in human exposure (EFSA, 2006; Pfohl-Leszkowicz and Manderville, 2007; JECFA, 2001; NTP, 2014). OTA exposure of humans is most frequently associated with kidney toxicity, which is frequently observed in areas with high OTA exposure. Balkan endemic nephropathy (BEN) occurs in areas where contamination with OTA is prevalent, particularly in the Balkans. The distribution of BEN is linked to areas of high incidence and mortality from urinary tract tumors (NTP, 2014; EFSA, 2006; JECFA, 2001). From the epidemiological data available, the relationship between exposure to OTA and human cancer cannot be determined (NTP, 2014).

Bui-Klimke and Wu reviewed the studies on OTA and human health risk that included a measurement of urinary or dietary OTA from both diseased and healthy individuals (Bui-Klimke and Wu, 2014). Of 22 studies reviewed, only three met their inclusion criteria. Of those three, two found no statistically significant evidence of an association between OTA exposure and human health risk. The other study found an increased risk of nephritic syndrome at very high exposures to OTA (3.09 ng/mL mean urinary OTA level) in a case-control study assessing multiple potential adverse health effects in an Egyptian population. The risk in this latter study was statistically significant even though the sample size was very small (n=15).

Measurement of the blood/serum and/or urine concentrations of OTA can be used as markers of OTA exposure because of the long serum half-life and the renal elimination of OTA in humans (EFSA, 2006; Pfohl-Leszkowicz and Manderville, 2007). In a Scandinavian study the reported

serum mean OTA concentration varied from 0.18 to 0.21ng/mL in Norway and Sweden respectively, based on analysis of 406 blood donors. The mean serum concentration in Croatia, based on 983 samples, was 0.39 ng/mL (EFSA, 2006). OTA is also found in ~98 % of the urine samples obtained from an area in Bulgaria with BEN and ranged from 0.016 - 0.860 ng/mL (Pfohl-Leszkowicz and Manderville, 2007).

4.6.4 Hazard Information - Animal

4.6.4.1 Acute Lethality

The LD₅₀ depends on animal strain, animal sex and the route of administration (JECFA, 2001; Pfohl-Leszkowicz and Manderville, 2007). Oral LD₅₀ range from 0.2 mg/kg in dogs to 58 mg/kg in mice. Neonates are much more sensitive than adults; the neonatal rat oral LD₅₀ was reported as only 3.9 mg/kg, compared with 20-30 mg/kg for adult rats (JECFA, 2001). OTA is more toxic when injected ip compared to oral administration. For example, the rat ip LD₅₀ is 13 mg/kg. Parenteral LD₅₀ vary from 20 mg/kg (sc) in rabbits to 112 mg/kg in mice (ip).

4.6.4.2 Acute/Short-term Toxicity

No data regarding primary skin irritation or sensitization were reported in EFSA (2006) or JECFA (2001).

There are several short term toxicity studies in rodents, dogs, and pigs reported in JECFA (2001). Overall, the kidney is the main organ affected by OTA, and effects were seen at doses of about 1 mg/kg-day in rats treated by gavage or feed for two weeks. The NOELs ranged from <0.24 – 5 mg/kg-day. Other toxic effects included cardiac and hepatic histological abnormalities, aberration of coagulation factors, hemorrhage and thrombosis in the spleen, brain, liver, kidney and heart, lesions of the gastrointestinal tract and lymphoid tissues, and myelotoxicity in mice (JECFA, 2001; Pfohl- Leszkowicz and Manderville, 2007).

EFSA (2006) reported on one toxicity study with OTB. F344 rats were treated with OTB orally (manner not available) with either a single high dose (10 mg/kg), or with repeated doses (2 mg/kg-day for 2 weeks). The only indication of toxicity was following exposure to a single high dose of 10 mg/kg, where mitotic figures were found in the renal proximal tubule cells.

4.6.4.3 Repeated Dose Toxicity

There are numerous repeat dose rodent studies reported by JECFA (2001) and EFSA (2006). These studies indicate the key targets of OTA toxicity are the kidney and potentially the immune system (see below). The observed kidney effects include effects on both function (increased urine volume, proteinuria, impaired urinary transport of organic substances) and structure (necrosis of tubular cells, karyomegaly). The most sensitive species is the pig, for which JECFA

(2001) identified a LOAEL of 0.008 mg/kg-day based on renal toxicity, apparently following capsule dosing. However, as discussed in detail below, detailed review of the primary data raised questions about this LOAEL.

Wistar rats (15/sex/group) were fed OTA at 0, 0.2, 1, or 5 ppm in diet (reported by EFSA, 2006 to be equivalent to 0, 0.015, 0.075, or 0.37 mg/kg-day) for 90 days. Relative kidney weights were reduced at the two higher doses, and dose-related increases in renal karyomegaly were detected at all doses. Based on the results from this study, EFSA (2006) identified a LOAEL of 0.015 mg/kg-day for renal toxicity, and no NOAEL.

In a 90 day gavage study, F344 rats (15 rats/group) received 0, 0.0625, 0.0125, 0.25, 0.5, or 1 mg/kg-day (NTP, 1989). Final body weights were decreased in both males and females, with the males being more sensitive. OTA-induced lesions in the kidney were observed at all doses and characterized as degeneration and regeneration of the epithelium of the proximal convoluted tubules, with necrosis of moderate severity. The LOAEL in this study was 0.0625 mg/kg-day; no NOAEL was identified.

Based on the subchronic study, NTP (1989) conducted a 2-year study in rats (80/sex/dose, with interim sacrifices of 15/sex/dose at 9 and 15 months) gavaged with ochratoxin at 0, 0.21, 0.07, or 0.21 mg/kg-day for 5 days/week (equivalent to 0, 0.015, 0.050, and 0.150 mg/kg-day) for up to 2 years. Renal tubule lesions, including degeneration and hyperplasia, were seen at the two highest doses; the only effect at the low dose was karyomegaly in females. EFSA (2006) identified a NOAEL of 0.015 mg/kg-day from this study.

Elling and coworkers conducted a series of studies on the effects of OTA on pigs (strain unspecified). These studies were reported by JECFA (2001) and EFSA (2006), but there appear to be substantial disconnects between the data reported in the reviews and the data reported in the original publications; the reason for these disconnects is unclear. Further complicating the issue, JECFA (2001) lumped descriptions of multiple studies together, making it difficult to identify the basis for specific doses. In the interests of transparency, this text reports both the data as provided by the reviews, and the data based on the primary studies.

JECFA (2001) reported the data as follows:

In a series of experiments, groups of three to six sows were given feed containing ochratoxin A at a concentration of 0, 0.2, 1, or 5 mg/kg, equivalent to 0, 0.008, 0.04, and 0.2 mg/kg per day, for periods of 5 days, 8 or 12 weeks, or up to 2 years. Decreased renal function, nephropathy, and reduced renal enzyme activity were reported. Progressive nephropathy but no renal failure was seen in female pigs given feed containing 1 mg/kg for 2 years; no results were reported for male pigs (Krogh & Elling, 1977; Elling,

1979a,b, 1983; Elling et al., 1985; Krogh et al., 1988)⁵.

In a table, JECFA (2001) reported a LOAEL of 0.008 mg/kg-day for exposures of 5-90 days, based on Elling (1979) and Krogh et al. (1988). JECFA did not provide further details on the basis for the reported doses in mg/kg-day. EFSA (2006) used the data reported by JECFA to identify 0.008 mg/kg-day as a NOAEL in a 2-year study, and a LOAEL in a 90-day study. EFSA used the dose of 0.008 mg/kg-day as the basis for its tolerable weekly intake.

Elling (1979) reported on changes in activity of kidney proximal tubular enzymes in pigs exposed to 5 ppm in feed for 5 days (3 pigs and 3 controls), or 1 ppm for 90 days (3 pigs and 3 controls) or 2 years (6 pigs and 6 controls). Elling (1979) noted that the 5 ppm concentration corresponded to 0.4 mg/kg-day but did not provide ingested doses for the 1 ppm in feed used for the 90-day and 2-year studies. Krogh et al. (1979) appears to have reported on the histopathology from the same 90-day and 2-year study, but noted that a group of 9 pigs and 9 controls was exposed to 1 ppm, with an interim sacrifice at 90 days. Krogh et al. (1979) further reported that the mean amount of OTA “available to each pig” in the 90-day and 2-year studies was 0.0377 mg/kg body weight – presumably 0.0377 mg/kg-day. The implied relationship between the concentration in feed and the ingested dose based on the Krogh et al. (1979) data is consistent with that reported by Elling (1979), taking into account the difference in study durations.

Changes in kidney proximal tubular enzyme levels indicative of tubular atrophy were observed at all three durations (Elling, 1979), but the magnitude of the change was not reported. In addition, increased urinary glucose excretion and decreased ability to concentrate urine occurred within a few weeks. Pigs sacrificed at 90 days had degeneration of the proximal tubules, tubular atrophy and interstitial fibrosis (Krogh et al., 1979). The pathology was more advanced at 2 years, and included interstitial fibrosis and tubular atrophy (Krogh et al., 1979). Thus, the results of Elling (1979) and Krogh et al. (1979) appear to identify a LOAEL of 0.04 mg/kg-day for a 90-day feeding study.

Krogh et al. (1988) provided OTA to pigs in gelatin capsules containing 0, 0.25, or 1.17 mg for 5 weeks, and conducted renal biopsies at 1, 3, and 5 weeks. Adding additional confusion, the doses were noted as corresponding to 0, 0.2, and 1 ppm, and the data tables used the ppm designations. Krogh et al. (1988) did not provide a conversion to dose in mg/kg-day, and the animal weights and feed/animal varied considerably. Therefore, for the purposes of this report, the conversion provided by Krogh et al. (1979) can be used, that 1 ppm ~ 0.04 mg/kg-day.

⁵ Based on the abstracts, Krogh and Elling (1977) is a general review, and Elling et al. 1985 is a 5-day study. The Elling 1979b reference cited by JECFA (2001) was an abstract only, and so was not further pursued, and the abstract for Elling (1983) could not be located.

Based on this conversion, the doses were 0, 0.008, and 0.04 mg/kg-day. Thus, this study appears to be the basis of the LOAEL of 0.008 identified by JECFA (2001), although the study duration is much shorter, and dosing was via capsule, not feed. The authors reported a dose-dependent decrease in renal function, based on the ratio of maximal tubular excretion of para-aminohippurate and clearance of inulin, although neither parameter was individually changed. Increased glucose excretion was also noted. Dose-related changes in enzyme levels in the tissue biopsy samples were also noted, suggestive of tissue damage. Thus, the data in this study appear to identify a LOAEL of 0.008 mg/kg-day, based on capsule dosing. However, as noted in the context of the carcinogenicity data, bolus dosing may result in substantially higher toxicity of OTA than seen with dosing in feed.

In a small study with male rats given OTA at 0.14-2 mg/kg-day by gavage for 56-84 days, decreased kidney enzyme activity and increased urinary enzyme activity were reported (JECFA, 2001); there was no NOAEL. In weanling rats given OTA at 0 or 100 mg/rat in sodium bicarbonate for 8 weeks, reported by JECFA (2001) as 1.25 mg/kg-day, blood glucose levels were elevated (2X above controls), and liver carbohydrate and glycogen concentrations were reduced.

Neurotoxicity (brain lesions and altered enzyme levels) was seen in rats given oral doses of 0.12-0.29 mg/kg-day for 1-6 weeks, 0.07 mg/kg-day in rats treated by gavage for 28 days, and 3 mg/kg-day in mice. Brain lesions were also seen in the fetuses of rats administered 0.5 mg/kg-day and rabbits administered 0.05 mg/kg-day during gestation. These studies show that neurotoxicity is associated with OTA exposure, but the doses required are approximately six times higher than those required for renal toxicity (EFSA, 2006).

A dietary study with male Wistar rats fed OTA at 4 ppm in feed (about 0.37 mg/kg-day, based on a food factor of 0.92) for 30 days resulted in altered levels of thyroid hormones, testosterone, and prolactin (Kumar et al., 2011).

4.6.4.4 Developmental and Reproductive Toxicity

OTA can cross the placenta and is teratogenic in rat, mouse, hamster, and rabbit models at doses ranging from 0.1-1 mg/kg-day (EFSA, 2006; Malir et al., 2014; Wangikar et al., 2004a, 2004b; JECFA, 2001).

Administration of OTA to Wistar rats by gavage at doses of 0, 0.125, 0.25, 0.50, or 0.75 mg/kg-day on GD 6 to 15 resulted in a dose dependent increase in skeletal and visceral anomalies of the fetus (Wangikar et al., 2004a, 2004b; EFSA, 2006). The effects were statistically significant at 0.50 mg/kg-day and higher. Statistically significant decreases in maternal weight *gain* were seen at 0.25 mg/kg-day at higher, so the fetal effects may have been secondary to maternal toxicity.

Developmental toxicity was also seen in a study of pregnant CBA mice given a single dose of OTA by gavage at 0, 1, 2, or 4 mg/kg-day GD 8 or 9, or 4 mg/kg-day 2 days prior to mating and GD 2, 4, 6, 7, 10, and 14 (JECFA, 2001). JECFA (2001) also stated that “no mention was made of maternal toxicity.” Observed effects included decreased prenatal survival (4 mg/kg-day), and craniofacial anomalies at all doses when treated on GD 9 (JECFA, 2001).

Developmental teratogenic effects (skeletal and visceral malformations) were also reported in Wistar rats given a single sc dose of 1.75 mg/kg-day on GD 6, and in golden hamsters administered a single dose of OTA by ip (2.5-20 mg/kg) on one of GD 7 through 10. Other rodent studies show reduced embryo growth and other embryotoxicities with craniofacial abnormalities being some of the most commonly observed toxic effects (Malir et al., 2014).

OTA inhibits 3-hydroxysteroid dehydrogenase activity and reduces ovarian steroidogenesis in rats. OTA also inhibits testosterone secretion *in vitro* and *in vivo* in rats (Malir et al., 2014). In experimental animals, OTA is toxic to the testis, reducing fertility. For example, male albino mice given OTA orally at 0.05 or 0.1 mg/day for 45 days had dose-dependent decreases in sperm counts, sperm mobility, sperm viability and fertility rate. Pigs exposed to OTA by feed at 0.02 or 0.04 mg/day had decreased sperm production and semen quality (Malir et al., 2014).

4.6.4.5 Genotoxicity

OTA is considered to be negative in conventional assays for genotoxicity (JECFA 2001, EFSA 2006, Pfohl-Leschowicz and Manderville, 2007) at both the gene and chromosome levels. OTA was negative in conventional mutation assays in *S. typhimurium* and *E. coli* but was positive in specialized assays conducted with conditioned medium derived from OTA-exposed primary rat hepatocytes or with kidney microsomes (EFSA, 2006), suggesting that standard exogenous metabolic activation systems may not produce the relevant genotoxic metabolite. A slight but significant increase in gene mutation was seen with OTA in mouse lymphoma cells and in Chinese hamster V79 cells both in the absence and presence of rat kidney microsomes; the results were considered to be consistent with oxidative stress-related mutagenesis. Positive results in the presence of arachidonic acid or NADPH cofactors suggested that OTA undergoes bioactivation by both prostaglandin H synthase and CYP450 (EFSA, 2006).

The data were less consistent for tests of chromosome damage (EFSA, 2006). Results were negative for chromosome aberrations in Chinese hamster V79 cells (with and without rat liver S9 fraction), in human renal (ACHN) and bronchial (W126) cell lines, and in a Chinese hamster epithelial liver cell line (CHEL) that retains metabolic competence. OTA was also negative for micronucleus induction in cytochalasin B blocked human lymphocytes in the presence and absence of rat kidney S9. However, increased chromosome aberrations were seen in bovine lymphocytes and increased micronuclei were seen in Syrian hamster embryo (SHE) cells and in ovine seminal vesicle (OSV) cells (which lacking mono-oxygenase activity but express high

levels of prostaglandin H synthase)(EFSA, 2006), and in primary kidney cells from rats and humans (Pfohl-Leszkowicz and Manderville, 2007).

In kidney cells, the target of OTA toxicity, OTA induced single-strand DNA breaks in a concentration-dependent manner and the addition metabolic activation enzyme system significantly increased the response (JECFA 2001, EFSA 2006, Pfohl-Leszkowicz and Manderville, 2007). Mixed results have also been seen in assays for DNA damage, such as unscheduled DNA synthesis and sister chromatid exchange.

Overall, although EFSA (2006) considered the data regarding the genotoxicity of OTA to be mixed and controversial, a clearer picture is emerging from more recent testing with non-standard metabolic activation systems. These results indicate that OTA causes gene mutations in the presence of specific metabolic activating systems, such as kidney microsomes and prostaglandin H synthase. The data also suggest that OTA can be clastogenic under similar activating conditions, although the absence of an effect in the human renal cell line is puzzling.

4.6.4.6 Carcinogenicity

IARC (1983) has classified OTA as a possible human carcinogen, group 2B. The National Toxicology Program Report on Carcinogens has stated “Ochratoxin A is reasonably anticipated to be a human carcinogen based on sufficient evidence of carcinogenicity from studies in experimental animals” (NTP 2014). This conclusion is supported both by cancer studies in experimental animals and from human epidemiological studies (JECFA, 2001).

Groups of 80 F344/N rats of each sex and dose group were administered OTA by gavage in corn oil at 0, 0.021, 0.070, or 0.210 mg/kg-day for 5 days/week (equivalent to 0, 0.015, 0.050, and 0.150 mg/kg-day) for up to 2 years (NTP, 1989). Groups of 15 rats/sex/dose were sacrificed at 9 and at 15 months, and the remaining survivors were sacrificed at 2 years. Ochratoxin was carcinogenic in both males and females. The NTP concluded that there is clear evidence of carcinogenic activity of OTA for male rats, based on increased incidences of kidney tubular cell adenomas (1/50, 1/51, 6/51, 10/50) and tubular cell carcinomas (0/50, 0/51, 16/51, 30/50). There was also clear evidence of carcinogenic activity for female rats, based on increased incidences of kidney tubular cell adenomas (0/50, 0/51, 1/50, 5/50) and tubular cell carcinomas (0/50, 0/51, 1/50, 3/50) and increased incidences and multiplicity of fibroadenomas of the mammary gland (17/50, 23/51, 22/50, 28/50).

Two dietary studies in rodents are among the additional studies that provide evidence that OTA is carcinogenic in rodents. The first is a two-year dietary study in which male F344 rats (controls = 30, treated = 64 animals) were provided 0 or 0.3 mg/kg-day OTA (calculated by the authors) until they reached 333 g, after which the rats received 0.1 mg/kg-day (Mantle et al., 2005). An increased incidence of renal tumors was seen. The authors suggested that the potency of OTA

was substantial lower when it was ingested in the diet as compared to oral gavage dosing in the NTP study, although a direct comparison was not possible due to gaps in the published NTP data and the different dosage regimens. The OTA in this study contained OTB at 5-10%, but that would not have affected the results, given the much lower toxicity of OTB.

Renal and hepatic tumors have been reported in ddY mice fed OTA at 40 ppm (equivalent to 5.6 mg/kg-day) for 44 weeks, followed by 5 weeks on a basal diet (JECFA, 2001). In another study, B6C3F1 mice (50/sex/dose) were fed OTA at 0, 1 or 40 ppm for 24 months (JECFA, 2001). The test compound contained about 84% OTA, 7% OTB, and 9% benzene. Benign and malignant renal tumors were seen in male mice, but not female mice, at the high dose.

4.6.4.7 Immunotoxicity

OTA can have effects on the immune system, inhibiting the proliferation of B and T lymphocytes, inhibiting the activity of killer cells in mice. Exposure of female Balb/c mice to OTA decreased the proportion of mature CD4+ or CD8+ lymphocyte cells, suppressed antibody production and the number of thymocytes (Pfohl-Leszkowicz and Manderville, 2007).

Immune function was affected (included altered natural killer cell activity) at all OTA doses tested in male Wistar rats treated with OTA by gavage for 28 days at 0, 0.05, 0.15, or 0.45 mg/kg-day. In female SPF Wag rats given 0, 0.07, 0.34 or 1.68 mg/kg-day in feed for 28 days, immune effects included a dose-related reduction in the splenic T-cell fraction and decreased IgG levels at 0.34 mg/kg-day and above (EFSA, 2006).

Immunotoxicity was also observed in limited studies with rats given oral doses of 0.05-0.1 mg OTA/kg-day for 28-35 days. The lowest immunotoxic dose of OTA is higher than the dose leading to measurable renal changes (EFSA, 2006).

4.6.4.8 In Vitro Studies and Data Related to Mode of Action

The accumulation of OTA in the kidney has been attributed to the binding of OTA to specific organic ion transporter proteins. Although the specific transporters have not been identified, interspecies and sex-related differences in sensitivity to OTA may be to variations in the transport mechanisms and cellular uptake by renal cells (EFSA, 2006).

A number of studies *in vitro* coupled with some data from *in vivo* investigations provide some clues to possible mode of action for the toxic effects of OTA exposure. The genotoxicity of OTA requires bioactivation, indicating that metabolism of OTA to reactive metabolite(s) is likely required (Pfohl-Leszkowicz and Manderville, 2007).

Reactive metabolites can bind covalently to DNA to form DNA adducts. OTA-DNA adducts have been reported in mice and rats treated with OTA. OTA-DNA adducts were detected *with in vitro* experiments using metabolic systems isolated from rat and mouse liver and kidney (Pfohl-

Leszkowicz and Manderville, 2007; EFSA, 2006; JECFA, 2001). However, the evidence for the formation of OTA-DNA is controversial because the OTA-DNA adducts have not been structural characterized and the activation enzyme(s) have not been fully determined.

Results from *in vitro* and *in vivo* studies suggest OTA is activated in the kidney, the target organ, by peroxidases, such as PHS (Pfohl-Leszkowicz and Manderville, 2007). OTA is also metabolized to quinones that can initiate peroxidation, leading to increases in reactive oxygen species. This increases cellular oxidative stress, which can also lead to DNA damage and can enhance apoptosis.

Table 17. Summary of OTA Toxicity Data

Study Type	Route, Species (duration)	References
Toxicokinetics	Studies in rodents, pigs and humans <i>in vivo</i> , plus numerous <i>in vitro</i> studies	JECFA, 2001
Acute lethality	≈7 oral studies in mouse, rat, rat neonate, dog, pig (LD50 = 0.2-58 mg/kg); 2 ip studies in mouse and rat (LD50 = 13-40 mg/kg); 2 iv in mouse and rat (LD50 13-34 mg/kg)	JECFA, 2001
Acute/short-term toxicity	1 dietary study in rats for 14 days; ≈6 gavage studies in rat, dog and pig for 3-14 days; 2 ip studies in the rat for 5-7 days OTB: 1 oral study in rats with a single dose or repeated doses for 2 weeks	JECFA, 2001 EFSA, 2006
Primary Irritation	None identified	
Sensitization	None identified	
Repeated dose toxicity	2 dietary studies: 1 in the rat for 90 days, 1 in the pig for 8-12 weeks. 4 gavage studies in rats: 1 for 56-85 days; 1 for 16 days; 1 for 91 days; 1 for 8 weeks	EFSA 2006 NTP, 1989
Developmental	2 by gavage in mice: 1 single dose by gavage on day 8 or 9 or 2 days before mating and on days 2,4,6,7,19 and 14 of gestation in mice; 1 on day 8 of gestation in mice. 5 by gavage in rat; 2 repeated doses on days 8-11, 8-13 or 8-15 in of gestation; 1 single dose on days 8 and 9 or days 8-10 in rats; 1 on days 11-14 of gestation; 1 by oral administration in pregnant rats on days 11-14.	Wangikar et al., 2004a, 2004b EFSA, 2006
Reproductive	No standard reproductive toxicity assays. Several studies found male reproductive effects, including 1 oral in albino mice, and 1 feeding study in pigs	Malir et al., 2014
Genotoxicity	Numerous studies <i>in vitro</i> : Multiple gene mutation assays in bacteria and mammalian cells using a variety of activation systems; multiple chromosome aberration	JECFA, 2001

Study Type	Route, Species (duration)	References
	and micronucleus assays in a variety of cell lines; multiple assays for DNA damage - unscheduled DNA synthesis, DNA strand break, sister chromatid exchange <i>In vivo</i> : 2 chromosomal aberration, 1 sister chromatid exchange, 2 DNA damage	
Carcinogenicity	1 gavage and 1 feeding in rats for 2 years, 4 feed studies in mice: 1 for 44 weeks; 2 studies at 70 weeks; 1 for 24 months	NTP, 1989 EFSA, 2006

4.6.4.9 Lowest Hazard Endpoints

The most prominent adverse effect of OTA is on the kidney in all animals species tested with pigs identified to be the most sensitive species. JECFA (2001) and EFSA (2006) identified a LOAEL of 0.008 mg/kg-day, based on “decreased renal function and nephropathy” in a 90 day feeding study. However, closer evaluation of the data indicates that this LOAEL resulted from capsule dosing, which may result in higher toxicity. Based on evaluation of the original studies, it appears that the LOAEL from the 90-day and 2-year pig feeding study was 0.04 mg/kg-day, based on changes in renal enzymes and kidney histopathology; no NOAEL was identified (Elling, 1979; Krogh et al., 1979). In Wistar rats exposed via the diet, a LOAEL of 0.015 mg/kg-day was identified based on renal karyomegaly, with no NOAEL (EFSA, 2006). In F344 rats exposed via gavage, the 90-day LOAEL was 0.0625 mg/kg-day, while 0.015 mg/kg-day was a NOAEL for kidney toxicity in the 2-year study (NTP, 1989; EFDA, 2006), indicating Wistar rats are more sensitive than F344 rats. The kidney was also the most sensitive target in short-term studies. Effects were seen at doses of about 1 mg/kg-day in rats treated by gavage or feed for two weeks; NOELs ranged from <0.24 – 5 mg/kg-day

Other toxic effects include immunotoxicity, neurotoxicity and teratogenicity; all at higher doses than does required for renal toxicity. For example, immune function (altered natural killer cell activity) was altered in male Wistar rats treated with OTA by gavage for 28 days at 0.05 mg/kg-day and higher, but no NOAEL was identified.

No data regarding primary skin irritation or sensitization were reported in EFSA (2006) or JECFA (2001).

The National Toxicology Program Report on Carcinogens has stated “Ochratoxin A is reasonably anticipated to be a human carcinogen based on sufficient evidence of carcinogenicity from studies in experimental animals” (NTP, 2014). This conclusion is supported by both cancer studies in experimental animals and from human epidemiological studies (JECFA, 2001). IARC (1983) has classified OTA as a possible human carcinogen, group 2B. Although the mode of

action has not been definitively determined, the data indicate that OTA is genotoxic under conditions where it is metabolized to reactive metabolite(s).

Table 18. Lowest Hazard Endpoints for OTA

Species/ Route	Duration	NOAEL/ LOAEL	Effect/Comments	Citation
Pigs/feed	90 days, 2 years	No NOAEL LOAEL 0.04 mg/kg-day	Renal toxicity – changes in renal enzymes kidney histopathology	Elling, 1979; Krogh et al., 1979
Wistar rat/feed	90 days	No NOAEL LOAEL 0.015 mg/kg-day	Renal toxicity-karyomegaly	EFSA, 2006
Pigs/capsule	5 weeks	No NOAEL LOAEL 0.008 mg/kg-day ⁶	Changes in kidney function	Krogh et al., 1988
Wistar rat/gavage	28 day	No NOAEL LOAEL 0.05 mg/kg-day	Immunotoxicity	ECHA, 2006
Wistar rat/gavage	GD 6-15	Maternal NOAEL 0.125 mg/kg-day, LOAEL 0.25 mg/kg-day Developmental NOAEL 0.25 mg/kg-day; LOAEL 0.5 mg/kg-day	Maternal – decreased body weight gain; Developmental toxicity – malformations, may have been secondary to maternal toxicity.	Wangikar et al., 2004a, 2004b; EFSA, 2006
			Reasonably anticipated to be a human carcinogen	NTP, 2014

4.6.5 References

Bui-Klimke, T.R., Wu, F. 2014. Ochratoxin A and human health risk: A review of the evidence. Crit Rev Food Sci Nutr. doi: 10.1080/10408398.2012.724480 (epub ahead of print).

EFSA (European Food Safety Authority). 2006. Opinion of the Scientific Panel on Contaminants in the Food Chain on a Request from the Commission related to Ochratoxin A in Food. The EFSA Journal 265, 1-56.

Elling, F. 1979. Ochratoxin A-induced mycotoxic porcine nephropathy: Alterations in enzyme activity in tubular cells. Acta Path Microbiol Scand. 87, 237-243.

⁶ JECFA (2001) and EFSA (2006) erroneously attributed this LOAEL to a 90-day feeding study

JECFA (Joint FAO/WHO Expert Committee on Food Additives). 2001. Ochratoxin A. IPCS/InChem. Available at:
<http://www.inchem.org/documents/jecfa/jecmono/v47je04.htm#2.2.3>

Krogh, P., Elling, F., Friis, C., Hald, B., Larsen, A.E., Lillehoj, E.B., Madsen, A., Mortensen, H.P., Rasmussen, F., Ravnskov, U. 1979. Porcine nephropathy induced by long-term ingestion of ochratoxin A. *Vet Pathol.* 16(4), 466-475.

Krogh, P., Gyrd-Hansen, N., Hald, B., Larsen, S., Neilsen, J.P., Smith, M., Ivanoff, C., Meisner, H. 1988. Renal enzyme activities in experimental ochratoxin A-induced porcine nephropathy: Diagnostic potential of phosphoenolpyruvate carboxykinase and gamma-glutamyl transpeptidase activity. *J Toxicol Environ Health.* 23, 1-14.

Kumar, S.N., Telang, A.G., Singh, K.P., Jain, A.K., Afroz, M., Patil, R.D. 2011. Experimentally induced toxicity of Ochratoxin A and Endosulfan in male Wistar rats: A hormonal disorder. *J Anim Vet Adv.* 10(13), 1750-1755.

Malir, F., Ostry, V., Pfohl-Leszkowicz, A., Novotna, E. 2013. Ochratoxin A: developmental and reproductive toxicity-an overview. *Birth Defects Res B Dev Reprod Toxicol.* 98(6), 493-502.

Mantle, P., Kulinskaya, E., Nestler, S. 2005. Renal tumourigenesis in male rats in response to chronic dietary ochratoxin A. *Food Addit Contam.* 2005(Suppl. 1), 58-64.

NTP (U.S. National Toxicology Program). 1989. Toxicology and Carcinogenesis Studies of Ochratoxin A (CAS No. 303-47-9) in F344/N Rats (Gavage Studies). Technical Report Series No 358. NTIS Publication No. PB90- 219478/AS. National Toxicology Program. Research Triangle Park, NC and Bethesda, MD. pp.142.

NTP (U.S. National Toxicology Program). 2014. Ochratoxin A. Report on Carcinogens, Thirteenth Edition. Department of Health and Human Services, National Toxicology Program. Research Triangle Park, NC.

Pfohl-Leszkowicz, A., Manderville, R.A. 2007. Ochratoxin A: An overview on toxicity and carcinogenicity in animals and humans. *Mol Nutr Food Res.* 51, 61-99.

Vettorazzi, A., Gonzalez-Peñas, E., Lopez de Cerain, A. 2014. Ochratoxin A: A review of analytical methods and studies in rat model. *Food Chem Toxicol.* 72, 273-288.

Wangikar, P.B., Dwivedi, P., Sinha, N. 2004a. Effect in rats of simultaneous prenatal exposure to ochratoxin A and aflatoxin B1. I. Maternal toxicity and fetal malformations. *Birth Defects Res B Dev Reprod Toxicol.* 71(6), 343-351.

Wangikar, P.B., Dwivedi, P., Sharma, A.K., Sinha, N. 2004b. Effect in rats of simultaneous prenatal exposure to ochratoxin A and aflatoxin B1. II. Histopathological features of teratological anomalies induced in fetuses. *Birth Defects Res B Dev Reprod Toxicol.* 71(6), 352-358.

4.7 Sterigmatocystin

4.7.1 Physical and Chemical Characteristics

Table 19. Physical and Chemical Characteristics of Sterigmatocystin

Characteristic		Reference
Molecular formula	C ₁₈ H ₁₂ O ₆	EFSA, 2013
IUPAC	((3aR,12cS)-3a,12c-dihydro-8-hydroxy-6-methoxy-7H-furo[3',2':4,5]furo[2,3-c]xanthen-7-one	EFSA, 2013
Cas No	10048-13-2	EFSA, 2013
Molecular weight	324.28 g/mol	EFSA, 2013
Melting Point	245-246 °C	EFSA, 2013
Physical State	Crystallizes as pale-yellow needles	EFSA, 2013
Solubility	Readily soluble in chloroform, as well as other organic solvents, such as methanol, ethanol and acetonitrile; low solubility in aqueous solutions (phosphate buffer) at different pH values.	EFSA, 2013
Decomposition	Stable (more than 95 % in chloroform) at 4 °C and at -20 °C for 30 days.	EFSA, 2013

Sterigmatocystin (STC) is a polyketide with a biosynthetic pathway in common with aflatoxin. STC is produced by several fungal species. *Aspergillus* species that synthesize STC include *A. flavus*, *A. parasiticus*, *A. versicolor* and *A. nidulans*, with *A. versicolor* being the most common source. Of the other genera addressed in this document, *Chaetomium* can also produce STC. There have been reports that some *Penicillium* species can produce STC, but a more recent assessment of molds for STC biosynthetic potential did not confirm this report (EFSA, 2013). Due to differences in the capacity to transform STC into O-methylsterigmatocystin, the direct precursor of aflatoxins B1 and G1, *A. nidulans* and *A. versicolor* can produce high amounts of

STC, while *A. flavus* and *A. parasiticus* produce low amounts of STC and higher amounts of aflatoxins.

4.7.2 Toxicokinetics

Limited data were located on the toxicokinetics of STC. The information available for STC suggests that it is rapidly absorbed following oral exposure, it is metabolized in the liver and lung by various cytochrome P450 (CYP) enzymes to form different hydroxymetabolites and a reactive exo-epoxide, and it is excreted as the parent compound and its hydroxylated metabolites via bile and urine.

Absorption data are limited to a study in a single monkey and a single study in rats (EFSA, 2013). The maximal rate of absorption [sic; it is not clear from the summary whether this should have reported as the extent, rather than rate of absorption] was 30% when a single vervet monkey was administered a single oral dose of 100 mg ¹⁴C-labelled STC (18.4 mg/kg) (EFSA, 2013). Although the rate of absorption was not calculated for rats given an oral bolus dose of 8 mg/kg of ¹⁴C-STC, peak plasma levels were reached after 30 minutes, while peak serum levels were reached after 3 hours in a single-dose study in rats at an unspecified dose. Plasma concentration-time curves from this bolus administration indicated significant differences between female and male rat in both age groups tested. Renal excretion accounted for 7.3-10.3% of the dose in the oral study, a finding that EFSA (2013) interpreted as indicating low absorption. However, the presence of biliary excretion and enterohepatic circulation suggests that absorption was much higher than the amount excreted in urine.

The distribution of STC has been investigated following oral treatment of rats with radiolabeled STC in single and repeated dose studies (EFSA, 2013). The half-life in rat serum was calculated to be 30 minutes. Radioactivity was widely distributed and concentrated mainly in liver, stomach, kidney, duodenum and lung and to a lesser extent in body fat, muscle tissue, testis, bone, and in rectum tissue. A high concentration was initially found in the stomach, consistent with histopathology lesions seen in rats chronically exposed to STC and gastric erosions observed in gerbils treated orally with STC (EFSA, 2013). In a repeated dosing study of rats administered 8 mg/kg STC in feed equally divided over 13 days, followed on the 14th day by an oral bolus administration of 8 mg/kg of ¹⁴C-STC, the highest level of tissue radioactivity was found in mature males and the lowest in immature females. Significant age differences were demonstrated, with tissue levels in different organs varying between mature and immature males and to a lesser extent between mature and immature females (EFSA, 2013).

Limited data on the metabolism of STC indicate that STC undergoes both phase I and phase II metabolism. Phase I metabolism of STC comprises CYP-mediated formation of a reactive epoxide, an exo-STC-1,2-oxide, as well as mono- and dihydroxylation reactions (IEFSA, 2013).

Several CYP isoforms are implicated in metabolically activating STC, based on *in vitro* tests using recombinant human CYP isoforms and *in vitro* genotoxicity tests (EFSA, 2013). Phase II metabolites reported in the literature include a glucuronide of STC and of monohydroxy-STC, as well as a sulfate conjugate of monohydroxy-STC and a glutathione (GSH) adduct of a monooxygenated STC (EFSA, 2013).

Excretion of STC following oral administration has been studied in rats and in one vervet monkey and via ip injection in rats (EFSA, 2013). In rats, most of the orally administered STC was eliminated in the feces (64–92 %), and about 10 % via urine, with elimination half-lives varying between 61.5 hours in immature females and 130 hours in mature male rats (EFSA, 2013). Ninety-six hours after the oral administration, the cumulative total elimination (urine and feces) exceeded 99 % in immature and mature males, and varied between 71.5 and 77.4 % in immature and mature females, respectively. In the vervet monkey, 50–80 % of the radioactively labeled oral dose was recovered in feces within 48 hours (EFSA, 2013). In rats treated ip with up to 16 mg/kg STC, only 3.1% of the administered dose was recovered in urine within 24 hours (EFSA, 2013). This low urinary excretion following ip dosing is consistent with the suggestion that urinary STC is not a good indicator of total absorbed STC.

4.7.3 Hazard Information- Human

Although no epidemiology studies were reported that evaluated the potential association of STC with any effects, EFSA (2013) reported on two studies conducted in China and other Asian countries that suggest a correlation between exposure to STC and the prevalence of gastric and liver cancer. In one study, a significantly higher rate of STC contamination of grains and higher levels of STC in grains were found in areas in China that had higher incidence of gastric cancers than in areas with a low incidence. STC and STC-DNA adducts were also reported in cancerous and precancerous tissues and in urine and blood samples of patients with liver and stomach cancer in China. Another study also reported STC in the blood serum of human patients, with significantly ($p < 0.001$) higher prevalence in patients with liver cirrhosis and liver cancer compared to controls. A strong correlation was also reported between the presence of alpha-fetoprotein (a tumor marker) and STC in patients with liver cancer, suggesting that STC may play a role in the pathogenesis of liver cancer. The data available do not provide conclusive evidence that the described liver and stomach cancers have indeed been induced by dietary exposure to STC but may support the use of STC concentration and DNA adducts in blood as biomarkers of recent exposure. Based on this, EFSA (2013) stated that the data cannot inform on the risk of STC.

4.7.4 Hazard Information - Animal

4.7.4.1 *Acute Lethality*

EFSA (2013) reported LD₅₀ studies in rats by the oral and ip routes and in monkeys by the ip route. Oral LD₅₀s in rats ranged from 120 to 166 mg/kg. LD₅₀ values in rats and monkeys exposed by ip injection were 32-65 mg/kg. According to EFSA (2013), the accuracy of the LD₅₀ values is questionable, since large volumes of solvent were used in order to overcome the low solubility of STC, and the authors did not quantify how the volume of solvent and the unabsorbed STC affected the observed oral LD₅₀. The liver and kidneys are the target organs of acute toxicity. Hyaline degeneration was observed in the kidney and necrosis and hemorrhages were noted in the liver and in the kidneys at lethal doses.

4.7.4.2 *Acute/Short-term Toxicity*

No data regarding primary skin or eye irritation and no data on sensitization were reported in EFSA (2013) or found in the literature.

No single-exposure oral or inhalation studies were located. A single intratracheal instillation of STC into Swiss Webster mice at 13 mg/kg lung weight resulted in increased mucus production and signs of alveolar inflammation at 12 hours after exposure.

Neonates may be more sensitive to the effects of STC than adults or other ages. A single sc injection of STC at doses as low as 0.5 mg/kg resulted in deaths within 5 days of treatment; almost all animals in the higher dose groups of 10-100 mg/kg died. Although similar studies via the same route are not available for adults, there is no reason to expect higher mortality via sc dosing than ip dosing. Only mortality was reported.

Male weanling rats were exposed to 100 ppm STC in feed (equivalent to about 15 mg/kg-day) and sacrificed in pairs every other week, starting after 2 weeks of exposure, for up to 16 weeks. After 2 weeks, the signs of toxicity included single cell necrosis in the liver and prominent Kupffer cell proliferation (EFSA, 2013).

Guinea pigs orally treated with STC at 4.2 mg/animal for two weeks (dose in mg/kg not reported) had significant weight loss (magnitude not reported) and hepatotoxicity (diffuse fatty degeneration of hepatocytes and focal necrosis) (EFSA, 2013). A proliferation of Kupffer cells and neutrophilic infiltration were reported in the necrotic foci. Treatment with STC also induced a significant decrease in complement activity in the guinea pigs; α 2- and β - globulins (major components of complement) were reduced in the absence of an effect on total serum protein. However, EFSA (2013) noted that these results do not necessarily indicate that STC has a

specific effect on complement, or is specifically immunotoxic, since components of complement are produced in the liver (and thus, the decrease may reflect general liver toxicity).

4.7.4.3 Repeated Dose Toxicity

No standard repeated-dose toxicity studies were identified for STC. Several carcinogenicity studies available for STC provided only very limited information on the potential of STC to cause systemic effects.

Wistar rats were exposed to STC at 100 ppm in feed for 16 weeks (average dose approximately 9 mg/kg-day, based on a food factor of 0.092) and sacrificed every other week starting after 2 weeks of exposure. After 12 weeks of exposure, the histopathology was characterized by foci of enlarged cells that progressed into large hyperplastic nodules surrounded by degenerating and necrotic hepatocytes, but with no substantial bile duct proliferation (EFSA, 2013).

When vervet monkeys received repeated gavage dosing of STC at 20 mg/kg every 14 days for 12 months (corresponding to 1.4 mg/kg-day), icterus and chronic hepatitis were reported after 4-6 months of treatment. The pathology progressed to aggressive hepatitis with further treatment. After 12 months of exposure, STC caused large hyperplastic nodules containing hepatocytes with pleomorphic nuclei throughout the liver (EFSA, 2013).

In a carcinogenicity study, male ACI/N rats (n=12-36) were fed a diet containing STC at concentrations of 0, 0.1, 1, or 10 ppm STC (reported by EFSA [2013] to correspond to 0.005, 0.05 and 0.5 mg STC/kg-day). Treatment with STC caused a clear dose-dependent increase in the incidence of central necrosis in the liver (Maekawa et al., 1979). Central necrosis, a non-preneoplastic change, is observed frequently in rats with chronic anemia or with toxic substances, and has been reported in rats when a high dose of STC was given orally (Maekawa et al., 1979). Maekawa et al. (1979) were uncertain whether or not all central necroses observed in the liver of the experimental groups were caused by STC. EFSA (2013) did not identify a noncancer effect level for this study.

4.7.4.4 Developmental and Reproductive Toxicity

No developmental or reproductive toxicity studies in mammals were identified for STC (EFSA, 2013) or in the open literature. Therefore, the potential of STC to cause developmental and/or reproductive toxicity cannot be evaluated.

4.7.4.5 Immunotoxicity

The immunotoxic potential of STC has also been investigated in two studies with pathogen-free BALB/c mice following single ip injections (EFSA, 2013). In one study, mice treated with 3 mg/kg had changes in levels of the cytokines tumor necrosis factor-alpha (TNF- α), interleukin

(IL)-12p35, and IL-6 that were consistent with immunosuppressive effects, although the presence of a dose-response relationship could not be assessed. In another immunotoxicity study, BALB/c mice treated ip with STC doses of 0.03 mg/kg and higher, but not 0.003 mg/kg, significantly altered the percentage of regulatory T cells (EFSA, 2013). EFSA (2013) noted that it is difficult to confirm these effects as specific immunotoxic effects, since information on systemic effects were not reported in the same studies, and the route was not environmentally relevant.

4.7.4.6 Genotoxicity

Genotoxicity of STC has been tested in a variety of assay systems in bacteria and mammalian cells *in vitro* and in rats *ex vivo* and *in vivo* (reviewed by EFSA [2013]). The weight of evidence for gene toxicity indicates that STC is mutagenic in both bacterial and mammalian cells after metabolic activation. It induces chromosomal damage both *in vitro* upon metabolic activation (micronuclei) and *in vivo* in experimental animals (chromosome aberrations). STC also induces UDS in metabolically competent primary hepatocytes from mice and rats. It also induces sister chromatid exchanges (SCEs, a measure of DNA damage) *in vivo*.

In vitro studies with purified DNA indicate that metabolically activated STC forms N⁷-guanyl DNA adducts, which are likely to be responsible for the mutagenic effects (EFSA, 2013). Thus, EFSA (2013) concluded that STC reacts with DNA to form adducts, which can result in mutations if not repaired.

4.7.4.7 Carcinogenicity

Five carcinogenicity studies in rats (4 dietary and 1 gavage) and two in mice (dietary), and one in non-human primate (oral) are available (reviewed by EFSA, 2013). The potential for STC to be carcinogenic has also been evaluated following intragastric, ip, sc, and dermal administration. The available data indicate that oral administration of STC resulted in premalignant and malignant lesions such as hepatocellular carcinoma (HCC), hemangiosarcomas in the liver, angiosarcomas in the brown fat, lung adenomas, and incidental findings in other organs. In addition, STC has also induced tumors after dermal, ip, and sc administration in a variety of species including Mongolian gerbil and monkey.

Maekawa et al. (1979) exposed 11-week-old male ACI/N rats (n=36 per experimental group and n=12 for control group) to STC in the diet throughout their life. Animals were exposed at a concentration of 0 (control), 0.1, 1, or 10 ppm (reported by EFSA [2013] to correspond to 0.005, 0.05 and 0.5 mg STC/kg-day). The animals were treated and observed for a maximum of 122 weeks. Mean survival times were 105, 93, 84, and 87 weeks for the 0, 0.005, 0.05 and 0.5 mg STC/kg-day groups, respectively. Large variation in individual survival times was observed. The incidences of tumor-bearing animals observed in the 0, 0.005, 0.05 and 0.5 mg STC/kg-day

groups were 7/11 (64%), 13/27 (48 %), 6/29 (21 %), and 10/26 (38 %) animals, respectively. Dose-related increases in liver tumors were observed, although the absolute incidence was low. HCC was observed only in a single high-dose animal (1/26, 4%). Hemangiosarcomas were seen in 1/29 (3%) and 3/26 (12%) rats in the 0.05 and 0.5 mg STC/kg-day groups, respectively, but not in the controls. A dose-dependent increase was observed in the incidence of hyperplastic foci or areas, and in central necrosis. The foci were described as consisting of normal or larger vacuolated or eosinophilic cells, not demarcated from surrounding cells and without disrupting the liver architecture. Hyperplastic nodules were observed only in the liver of 3/26 rats in the highest dose group.

In a second rat study, weanling rats were provided feed with STC produced by *Bipolaris* spp. on maize at concentrations up to 100 ppm for 6 months (calculated by EFSA [2013] to correspond to 0.5 mg/kg-day), then up to 150 ppm in feed (corresponding to approximately to 7.5 mg/kg-day) for another 6 months, followed by normal diet until the end of the experiment at 123 weeks. Treatment resulted in a dose-dependent increase in the incidence of HCC, accompanied by necrosis. Other rats in the same study were gavaged with doses up to 1.5 mg/rat for 5 days/week for 52 weeks (approximately 3 mg/kg-dose, or 2.1 mg/kg-day), and also developed HCC. In another study, male rats receiving a single gavage dose of 20 mg/kg STC once a week for 48 weeks developed nodular hyperplasia and foci in the liver sections (EFSA, 2013). A dose-dependent increase in HCC was seen in rats gavaged with STC at doses up to 1.5 mg/rat for 5 days/week (corresponding to up to 3.57 mg/kg-day) (EFSA, 2013). In another study, rats were fed *A. versicolor*-contaminated rice containing STC at doses up to 10 ppm in feed (corresponding to up to 0.5 mg/kg-day) for 96-101 weeks. Treatment resulted in reduced group survival and a dose-dependent increase in the incidence of hepatic tumors, with the tumors in most cases being classified as HCC and in some cases hemangiosarcomas (EFSA, 2013). The incidence of lung neoplasia was also increased in the dosed groups. Rats fed diets containing STC at 10 ppm feed (corresponding to up to 0.5 mg/kg-day) for 54 weeks developed HCC, but control animals did not develop any tumor (EFSA, 2013). Many of these studies were conducted with sample sizes as small as 10/group, but still exhibited clear dose-related increases after only a year of exposure, reflecting the carcinogenic potency of STC.

In one of the two 2-year carcinogenicity studies in mice, there was a dose-dependent increase in the incidence of lung neoplasia in both sexes when the animals were fed STC in the diet intermittently (2 weeks of treatment, followed by 2 weeks of uncontaminated diet) for 54-58 weeks at doses up to 5 ppm in feed (reported by EFSA [2013] to correspond to an average of 0.75 mg/kg-day) (EFSA, 2013). In the second mouse study (EFSA, 2013), female mice fed rice contaminated with STC at a dose of up to 120 ppm in feed (corresponding to up to 18 mg/kg-day) for 73 weeks developed a dose-dependent increase in the incidence of brown fat and in the incidence of lung neoplasia. In addition, the mice developed liver tumors (hepatic

hemangioendotheliomas and angiosarcoma), but these were not dose-related. Quantitative conclusions from the second study are limited by multiple adjustments made to the dosing level.

A long-term study was conducted with non-human primates dosed orally once per week with STC at doses up to 2.0 mg/kg (corresponding up to 0.29 mg/kg-day), but no final report was identified in the literature (EFSA, 2013). STC was considered a potent hepatocarcinogen, resulting in the development of HCCs, hepatic cholangiocarcinomas, and cholangiosarcoma. One monkey also developed renal cell carcinoma, but the numbers were too low to determine whether this was exposure-related. Extensive liver damage was noted in the surviving animals.

Rats treated dermally with STC for 70 weeks developed skin tumors (papillomas and squamous cell carcinomas) and HCC. Carcinogenicity of STC was also demonstrated in rats and mice after ip and sc administration (EFSA, 2013).

Overall, carcinogenicity of STC has been reported after oral (dietary and gavage), ip, sc, and dermal administration resulting in hepatocellular carcinomas, hemangiosarcomas in the liver, angiosarcomas in brown fat and lung adenomas.

4.7.4.8 *In vitro* Studies and Data Related to Mode of Action

Several *in vitro* and *in vivo* studies have been conducted to investigate the mode of action of STC for mutagenic effects as well as for other potential forms of toxicity. Various *in vitro* and *in vivo* investigations have also demonstrated that STC induces cytotoxicity, inhibits cell cycle progression and mitosis, and increases the formation of reactive oxygen species and lipid peroxidation (EFSA, 2013). The cytotoxicity occurs primarily via necrosis, and has been reported to be related to impairment of mitochondrial ATP synthesis (uncoupling of oxidative phosphorylation). Oxidative stress and lipid peroxidation as a secondary mechanism were also considered to play causal roles in cell damage and necrosis (EFSA, 2013). EFSA (2013) noted that many of the *in vitro* assays have been conducted with rather high STC concentrations, and so the relevance of the observed effects for the assessment of the potential adverse effects of low dose exposure to STC is limited.

Table 20. Summary of Toxicity Data of Sterigmatocystin

Study Type	Route, Duration	References
Toxicokinetics	1 single dose oral study in vervet monkeys, 1 single dose and 1 repeated dose dietary studies in rats, 1 ip study in rats, 6 <i>in vitro</i> studies	EFSA, 2013
Acute lethality	1 oral study in rats (LD ₅₀ =120-166 mg/kg for F and M); 1 ip	EFSA, 2013

Study Type	Route, Duration	References
	study in rats (M and F) and 1 in vervet monkeys (LD ₅₀ = 32-65 mg/kg)	
Acute/short-term toxicity	1 sc study in neonatal mice for 5 days; 1 feeding study in weanling guinea pigs for 2 weeks; 1 oral study in guinea pigs for 2 weeks; 2 single-dose ip immunotoxicity studies in mice.	EFSA, 2013
Primary Irritation	No data for skin or eye irritation	EFSA, 2013
Sensitization	No data available	
Repeated dose toxicity	1 dietary study in rats for 12 weeks; 1 intragastric study in vervet monkeys for 12 months; 1 dietary study in rats for 122 weeks	EFSA, 2013
Developmental	No data available	EFSA, 2013
Reproductive	No data available	EFSA, 2013
Genotoxicity	At least 5 bacterial gene mutation assays; 4 mammalian gene mutation assays; multiple DNA repair assays (bacterial, UDS, <i>in vivo</i> SCE); 2 <i>in vitro</i> micronucleus assays; 1 <i>in vivo</i> assay of chromosome damage	EFSA, 2013
Carcinogenicity	At least 4 dietary, 2 gavage studies in rats, many with relatively short exposure duration and extended follow up, 2 dietary studies in mice for 54-73 weeks, and 1 oral study in nonhuman primates; 1 dermal in rats; 2 sc (rats and mice), and 1 ip (rats)	Maekawa et al., 1979; EFSA, 2013

4.7.4.9 Lowest Hazard Endpoints

The primary targets of STC are the liver and, to a lesser extent, the kidney. Oral and parenteral studies in rats and mice for durations ranging from acute to chronic have shown liver necrosis. Hepatitis was also reported in a monkey study, and hyaline degeneration of the kidney in an acute lethality study. None of the short-term or subchronic studies tested more than one dose level, and so it is not possible to identify effect levels. However, adverse effects in the liver have been reported at oral doses as low as 15 mg/kg-day in rats for 2 weeks, and 1.4 mg/kg-day (based on the average dose under an intermittent dosing scenario) in monkeys for 12 months (EFSA, 2013).

It is unclear whether a lower effect level was identified in the chronic rat study by Maekawa et al. (1979). In this study, the incidence of hepatic necrosis and hyperplastic foci (a potential preneoplastic lesion) appeared to be higher in the low dose (0.005 mg/kg-day) than in the controls. This suggests that 0.005 mg/kg-day may be an adverse effect level for STC in a 2-year study, but no statistical analysis was conducted, and any such analysis would be limited by the small size of the control group (effective n=11).

Immunotoxic effects (altered cytokine levels, altered T cell levels) have been reported following ip injection, but no standard immunotoxicity studies have been conducted, and the study protocol was not adequate to differentiate between direct immunotoxicity and immunotoxicity secondary to systemic toxicity (EFSA, 2013).

No studies have investigated the developmental or reproductive toxicity of STC. Similarly, no studies were located investigating the potential for STC to cause primary skin or eye irritation and or sensitization.

STC forms DNA adducts and causes gene mutations in a wide range of *in vitro* and *in vivo* studies. The carcinogenicity of STC has been observed in many different species and test conditions. Perhaps due to the high carcinogenic potency, most of the studies were for less than the standard rodent testing period, and many used only small sample sizes. Despite these limitations, STC was clearly positive. The International Agency for Research on Cancer (IARC) has assessed the carcinogenic potential of STC (IARC, 1976, 1987) and concluded that oral exposure to STC produced lung tumors in mice and liver tumors in rats following oral administration. IARC also noted that in rats, dermal exposure to STC induced skin and liver tumors, and sarcomas were seen at the site of sc injection. IARC concluded that STC is “possibly carcinogenic to humans” (group 2B). STC has been associated with an increased prevalence of gastric cancers in Asia, but no causal association has been demonstrated.

For genotoxic carcinogens, EFSA uses a margin of exposure (MOE) approach, rather than calculating a tolerable daily intake. EFSA (2013) used the benchmark dose (BMD) approach to analyze the Maekawa et al. (1979) data. The other cancer studies were not considered appropriate for quantitative analysis, for reasons such as testing only one dose group, no clear dose-response, use of discontinuous dosing, or high mortality for reasons other than cancer. EFSA (2013) conducted the BMD modeling on liver hemangiosarcomas in the Maekawa et al. (1979) study, as the only tumor type with sufficient dose-response information. EFSA (2013) reported that, based on the adequate fitting models, the lowest BMDL₁₀ (lower 95% confidence limit on the dose corresponding to a 10% response) was 0.16 mg/kg-day, and the corresponding BMD₁₀ was 0.36 mg/kg-day. However, EFSA noted that the rats with hemangiosarcomas accounted for only 11% of the tumor-bearing animals in the study. The potency calculated for STC is several orders of magnitude lower than that for aflatoxin B₁, a related mycotoxin.

Overall, although the carcinogenic effects of STC are clear, there are gaps in the database regarding effect levels for acute and subchronic exposure. In addition, there are data gaps for developmental and reproductive toxicity.

Very limited data suggest that children may be more sensitive than adults to the liver effects of STC. People with liver or kidney damage would also be expected to be sensitive populations. Age- and sex-related differences in toxicokinetics were noted in rats, but these differences have not been connected to differences in toxicity, and the relevance to humans is unknown.

Table 21. Lowest Hazard Endpoints for STC

Species/ Route	Duration	NOAEL/ LOAEL	Effect/Comments	Citation
Rat/feed	2 weeks	No NOAEL ~15 mg/kg-day	Single cell hepatic necrosis. Single dose	EFSA, 2013

Species/ Route	Duration	NOAEL/ LOAEL	Effect/Comments	Citation
			tested	
Vervet monkey/ gavage	12 months	No NOAEL LOAEL 1.4 mg/kg-day	Chronic hepatitis after 6 months. Average dose under an intermittent dosing scenario (20 mg/kg every 14 days)	EFSA, 2013
			Possibly carcinogenic to humans	IARC, 1987

4.7.5 References

EFSA (European Food Safety Authority), 2013. Scientific Opinion on the risk for public and animal health related to the presence of sterigmatocystin in food and feed. EFSA Journal 11(6):3254, 81

IARC (International Agency for Research on Cancer). 1976. Monographs on the evaluation of carcinogenic risks to humans. Some naturally occurring substances, Volume 10. p. 245. International Agency for Research on Cancer.

IARC (International Agency for Research on Cancer), 1987. Monographs on the evaluation of the carcinogenic risks to humans. Overall evaluations of carcinogenicity: an updating of IARC Monographs Volumes 1 to 42. Supplement 7, 449 pp. International Agency for Research on Cancer.

Maekawa, A., Kajiwar, T., Odashima, S., Kurata, H. 1979. Hepatic changes in male ACI/N rats on low dietary levels of sterigmatocystin. Gann 70, 777-781.

4.8 Satratoxin

4.8.1 Physical and Chemical Characteristics

Stachybotrys chartarum (*Stachybotrys*) is a toxigenic black mold that can be isolated from indoor air of houses (Chung et al., 2003) and produces a wide range of mycotoxins and biologically active metabolites (Carey et al., 2012). *Stachybotrys* strains can be divided into two “chemotypes,” based on the toxins formed. One chemotype forms highly toxic macrocyclic trichothecene mycotoxins. The second does not make macrocyclic trichothecenes but does make less toxic atranones (dallabellane diterpenes) and simple trichothecenes (Pestka et al., 2008).

However, information was not located on which of the simple trichothecenes are made by the second chemotype.

Trichothecenes are sesquiterpenoids with a common 9, 10 double bond and a 12, 13 epoxide group; extensive variation exists relative to ring oxygenation patterns (Pestka et al., 2008). Trichothecene mycotoxins are also formed by toxicogenic species of grain fungi of the genera *Fusarium*, *Mycothecium*, *Trichoderma*, *Trichothecium*, *Verticimonosporium* and *Cephalosporium* (EFSA, 2008). Many of the trichothecenes for which toxicity data exist are formed by *Fusarium* species but do not appear to be formed by *Stachybotrys* species. These include the Type A trichothecenes [T-2 toxin (T-2), HT-2 toxin (HT-2), neosolaniol (NEO), diacetoxyscirpenol (DAS) and monoacetoxyscirpenol (MAS)] and Type B trichothecenes [deoxynivalenol (DON, also known as vomitoxin) and its 3-acetyl and 15-acetyl derivatives (3AcDON and 15AcDON, respectively), nivalenol (NIV) and fusarenon X (FusX)] (EFSA, 2008).

Stachybotrys specifically forms macrocyclic (or Type D) trichothecenes, including satratoxins G and H (SG and SH, respectively), roridin A, verrucarins A, roridin E, and verrucarins J (Bata et al., 1985; WHO, 1990; Kuhn and Ghannoum, 2003; McCormick et al., 2011; Pestka et al., 2008). Of the macrocyclic trichothecenes, limited information is available primarily on satratoxins G and H. Based on *in vitro* studies in lymphocytes and monocytes, the macrocyclic trichothecenes are considered more toxic than the simpler trichothecenes (Amuzie et al., 2010).

In addition to the trichothecenes, *Stachybotrys* can also produce other secondary metabolites, including trichoverrins, spirodrimanones and atranones (Pestka et al., 2008; Yike et al., 2005); cyclosporin (Kuhn and Ghannoum, 2003); stachylysin - a hemolytic protein (Yike et al., 2005; Pestka et al., 2008); stachybotrins, glucanases and staplabin (Terr, 2001); other proteinases (Yike et al., 2005; Pestka et al., 2008); glucans, and volatile organic compounds (Pestka et al., 2008). The specific compounds produced vary with the strain, isolate, and culture medium. Betancourt et al. (2013) evaluated volatile compounds emitted by seven toxigenic strains of *S. chartarum*. Most of the volatile compounds were alcohols, ketones, hydrocarbons, ethers and esters, with the compounds emitted by multiple strains including anisole and 3-octanone. It was also noted that several of the toxins are listed by only one publication, and it is not clear whether this reflects differences in nomenclature, mycotoxin identification, or other differences. Differences in the mycotoxins identified may also relate to challenges associated with isolating pure fungus cultures and differences in mycotoxins produced under different culture conditions. Even comparisons across related toxins can be a challenge. Toxigenic strains of *Stachybotrys* do not always produce the relevant toxins, and the levels of toxin production from the same strain may differ with culture conditions (Kuhn and Ghannoum, 2003). Thus, observations with toxins in culture or in isolation may not reflect environmental conditions.

In light of the many toxins produced by *Stachybotrys* and the fact that the effects of the *Stachybotrys* organism are attributed to its toxins, it is somewhat artificial to distinguish between

the effects of the toxins and the effects of the organism itself. However, this section addresses the effects of the primary toxins produced by *Stachybotrys* when studied in isolation, while effects of whole organism exposure are addressed in Section 3.11. See also the separate write-ups of cyclosporine in Section 4.4.

4.8.2 Toxicokinetics

Limited information exists on the kinetics of trichothecene uptake and disposition *in vivo* (Pestka et al., 2008). Probably based on information obtained from T-2 and, because of their lipophilic nature, trichothecenes are reported to be readily absorbed through the intestinal tract and the skin (Pestka et al., 2008; Wannecher and Wiener, 1997), but are only slowly absorbed when applied as a dust or powder (Wannemacher and Wiener, 1997). However, it was not clear whether these statements referred only to the simple trichothecenes or also relate to the macrocyclic trichothecenes.

The trichothecenes do not require metabolic activation to exert their toxic effects.

Trichothecenes are metabolized primarily in the liver, via deacetylation and de-epoxidation (hydrolysis) (Wannecher and Wiener, 1997). Trichothecenes, including the macrocyclic trichothecenes, bind to proteins, RNA and DNA (Wannecher and Wiener, 1997; Pestka et al., 2008).

Detailed information on the toxicokinetics of the macrocyclic trichothecenes or other factors that are produced by *Stachybotrys* is lacking in the literature. Some information, however, is available on the disposition of SG following intranasal exposure of mice (Amuzie et al., 2010). Since nasal instillation is not an environmentally relevant route of exposure, the specifics of absorption are not relevant, but this study does provide useful information on the systemic disposition of absorbed SG. In addition to the respiratory tract, SG is distributed to the kidney, spleen, thymus, and heart. Clearance is rapid, with a plasma half-life of 20 minutes, and a half-life in the thymus of 10 hours.

4.8.3 Hazard Information- Human

Cases of human toxicosis (stachybotryotoxicosis) have been reported after ingestion of *Stachybotrys*-contaminated foodstuffs (Johaning et al., 1996). Stachybotryotoxicosis has also been reported in farm workers and in people living in water-damaged homes with a heavy infestation of *Stachybotrys* (Wannemacher and Wiener, 1997; EPA, 2004). Satratoxin produced by *Stachybotrys* has been suggested as being in part responsible for this toxicosis. Although the macrocyclic trichothecenes produced by *Stachybotrys* are reported to be among the most potent protein synthesis inhibitors (Yike et al., 2005), dose-response information was not identified.

The simple tricothecenes are reported to cause skin irritation (erythema, edema and necrosis) in humans following direct contact (EPA, 2004), but it is not clear whether this has been observed for the macrocyclic tricothecenes.

Other effects of human exposure to *Stachybotrys* are addressed in Section 3.11.

4.8.4 Hazard Information - Animal

Macrocyclic tricothecenes are reported to be extremely potent biological modulators that cause numerous pathophysiologic effects in experimental animals and that could contribute to adverse human health effects (Pestka et al., 2008). Adverse effects have been reported in animals following ingestion (or inhalation) of feed contaminated with *Stachybotrys* (Pestka et al., 2008). The effects were reported to occur in two phases. The first phase consists of elevated body temperature, listlessness, epistaxis (bleeding from the nose), and intermittent hemorrhagic diarrhea, while the second phase involves a progressively worsening anemia, leukocytopenia (a decrease in the number of leukocytes in the blood), hemorrhage, and pulmonary congestion. Pestka et al. (2008) stated that these adverse effects are highly consistent with the known toxic effects of tricothecene mycotoxins. Information available on the more extensively studied Type A, T-2 tricothecene, indicates that dermal exposure to high concentrations can also lead to systemic toxicity and lethality, especially if the mycotoxin is dissolved in DMSO (Wannemacher and Wiener, 1997). Although no information is available on dermal toxicity of the macrocyclic tricothecenes produced by *Stachybotrys*, it can be assumed that they may also cause dermal toxicity similarly to T-2.

Atranones can also induce pulmonary inflammation (Pestka et al., 2008). Only limited information on atranones was identified, primarily via intratracheal instillation studies. These data indicated that the atranones varied in inflammatory potency.

4.8.4.1 Acute Lethality

No studies were identified that reported oral or dermal LD₅₀ values in animal species tested with single doses of satratoxins or other *Stachybotrys* metabolites. However, satratoxin H has an intraperitoneal LD₅₀ of 1.0 mg/kg in mice, while the intravenous LD₅₀ values in the mouse, rat and rabbit for verrucarins A range from 0.54 to 1.0 mg/kg and intravenous LD₅₀ in the mouse for roridin A was 1.0 mg/kg (Wannemacher and Wiener, 1997).

4.8.4.2 Acute/Short-term Toxicity

Although direct dermal contact with the simple tricothecenes is reported to cause skin irritation in humans (EPA, 2004), it is not clear whether this has been observed in animals. No studies were identified regarding the skin sensitization potential of satratoxins and other *Stachybotrys* metabolites that indicate that they act as skin sensitizers.

Although clear dose-response information was not identified for environmentally-relevant routes in the literature reviewed, some information can be gleaned from intranasal instillation studies. Carey et al. (2012) conducted studies in which adult male rhesus macaques were treated with SG via nasal instillation for 1 or 4 days. Observed symptoms included acute rhinitis, atrophy of the olfactory epithelium, and apoptosis of olfactory sensory neurons. The authors noted that although the high dose tested (about 20 µg) would require exposure to a large number (about 20 million) *Stachybotrys* spores, SG is also found in viable and nonviable fungi and fungal fragments, and the airborne concentrations of these materials may be much higher than that of airborne spores. The authors also suggested that if a single spore is deposited within the nasal tract, the local concentration of SG would be much higher than the average concentration.

Based on a study of a single intranasal instillation in mice, Pestka et al. (2008) reported a NOAEL and LOAEL for SG- induced nasal olfactory toxicity of 5 and 25 µg/kg, respectively, corresponding to 5–25 million spores/kg.

Hematotoxic effects have been associated with trichothecenes, and T-2 has been explored as a potential biological weapon (Pestka et al., 2008). The hematotoxic activity has been at least partially attributed to the blocking of protein, DNA and RNA synthesis, which results in lesions in rapidly dividing cells. It was not clear whether the macrocyclic trichothecenes also cause hematotoxicity; although they do bind to cellular macromolecules, it was unclear from the literature reviewed whether they also interfere with ribosome function,

4.8.4.3 Repeated Dose Toxicity

No repeated-dose standard or nonstandard toxicity studies were identified that investigated the potential of satratoxins or other *Stachybotrys* metabolites to cause systemic effects following oral or dermal exposure.

4.8.4.4 Developmental and Reproductive Toxicity

No guideline studies were identified that evaluated the potential of the satratoxins or the other *Stachybotrys* metabolites to cause reproductive and developmental effects following oral or dermal exposure. However, Kuhn and Ghannoum (2003) noted that ingestion of infected grain, liquid growth medium, or partially purified toxin caused a decrease in the number of pregnant animals, an increased frequency in dead, resorbed, or stunted fetuses, and decreased average litter size. Histopathological examination revealed uteroplacental hemorrhages. These fetal effects were reported in the absence of maternal toxicity. However, no quantitative or dose-response information was provided by Kuhn and Ghannoum (2003), and the authors noted that the relevance of the effects following oral exposure to the more likely route of inhalation

exposure is not known. The toxin potentially responsible for the observed effects was not identified.

4.8.4.5 Immunotoxicity

Information on the immunotoxic potential of the satratoxins or other *Stachybotrys* metabolites following *in vivo* exposure is lacking.

4.8.4.6 Genotoxicity

The epoxytrichothecene mycotoxins, including satratoxin H, are inactive both as base pair and as frame shift mutagens in several independent assays with the *Salmonella*/microsome system (Sorsa et al., 1980). Satratoxin H was also negative in the sex-linked recessive lethal test of *Drosophila melanogaster*; however, feeding parental flies fed with satratoxin H resulted in a slight, but significant increase in both maternal and paternal non-disjunction in the F1 progeny (Sorsa et al., 1980). Pestka et al. (2008) reported that the macrocyclic trichothenes or other factors from *Stachybotrys* also have the potential to damage DNA, since aqueous extracts of trichothecene-producing *Stachybotrys* spores induced DNA fragmentation in developing rat lung fibroblasts.

4.8.4.7 Carcinogenicity

No chronic toxicity/carcinogenicity studies were available for the satratoxins or other minor *Stachybotrys* metabolites following oral or dermal exposures.

4.8.4.8 In vitro Studies

Studies in numerous experimental systems both *in vivo* and *in vitro* indicate that satratoxins inhibit protein synthesis and cause direct cellular cytotoxicity (Terr, 2001). This is reported to be mediated through apoptosis by activation of protein kinases (Terr, 2001). The proteinases are reported to have the potential to induce inflammation via protease-activated receptors; to hydrolyze several collagens (types I, VI, and X), pulmonary proteinase inhibitors and several neuropeptides; and contribute to degradation of extracellular matrix proteins either directly via collagenolytic activity or indirectly via changes in the proteinase–antiproteinase balance (Pestka et al., 2008). The polysaccharide (1 → 3)- β -D-glucan has been linked to the development of inflammatory reactions, shown to exacerbate the inflammatory effects of dust on the upper airways of human volunteers, and to evoke upper respiratory tract symptoms and induction of cytokine production by blood monocytes in humans (Pestka et al., 2008). The spirocyclic drimanes have the potential to inhibit proteolytic enzymes, disrupt the complement system, inhibit TNF- α release, endothelin receptor antagonism, and stimulate plasminogen, fibrinolysis, thrombolysis, and cytotoxic and neurotoxic effects (Pestka et al., 2008).

Chung et al. (2003) reported on the effects of various concentrations of SG, SH, isosatratoxin F, roridin A, and verrucarin A on production of proinflammatory cytokines, tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6), when murine macrophage cells were treated *in vitro* with these mycotoxins in the presence or absence of suboptimal concentration of lipopolysaccharide. At low concentrations, these macrocyclic trichothecenes superinduced expression of TNF- α , whereas higher concentrations of these toxins were cytotoxic and reduced cytokine production.

Table 22. Summary of Toxicity Data for Satratoxin

Study Type	Route, Duration	References
Toxicokinetics	1 satratoxin H i.p study in mice (LD ₅₀ = 1.0 mg/kg), 1 verrucarin A i.v. study in mice, 1 in rat, and 1 in rabbit (LD ₅₀ s = 0.54-1.0 mg/kg); 1 roridin A i.v. study in mice (LD ₅₀ = 1.0 mg/kg)	Wannemacher and Wiener, 1997
Acute lethality	Limited information	
Acute/short-term toxicity	Limited information	
Primary Irritation	Limited information	
Sensitization	No data available	
Repeated dose toxicity	No dose-response data	
Developmental	No dose-response data	
Reproductive	No dose-response data	
Immunotoxicity	1 in vitro study using murine macrophage cells	Chung et al., 2003
Genotoxicity	Unspecified number of bacterial gene mutation assays; 1 sex-linked lethal test (mutations) of <i>Drosophila</i> ; 1 non-disjunctionisex chromosome loss in offspring of <i>Drosophila</i> ; in vitro assay of DNA damage	Sorsa et al., 1980; Pestka et al., 2008
Carcinogenicity	No data available	

4.8.4.9 Lowest Hazard Endpoints

Although there are human and animal data on the effects associated with stachybotrystoxicosis (see Section 3.11), the data attributing specific effects to specific *Stachybotrys* toxins are much more limited, and so no table of effect levels is included. Data are clearest on the association between respiratory tract toxicity and inhalation exposure. It has been reported that direct contact with the trichothecenes causes skin irritation in humans (EPA, 2004), but no studies were located investigating the potential for the macrocyclic trichothecenes to cause primary skin or eye irritation and or sensitization under controlled conditions. The macrocyclic trichothecenes do not appear to cause point mutations, but they do cause DNA strand breaks and induce genes indicative of DNA damage. No information exists on the potential carcinogenic effects of the macrocyclic trichothecenes. Although the trichothecenes are reported to be reproductive and

developmental toxicants (Kuhn and Ghannoum, 2003), the supporting data are weak and there are no dose-response data.

Overall, the macrocyclic trichothecenes and other metabolites produced by *Stachybotrys* have the potential to cause systemic effects, but dose-response data are not available to determine the effect levels.

There are no data that suggest that children may be more sensitive than adults to the liver effects of the macrocyclic trichothecenes.

4.8.5 References

Amuzie, C. J., Islam, Z., Kim, J. K., Seo, J. H., Pestka, J. J., 2010. Kinetics of satratoxin G tissue distribution and excretion following intranasal exposure in the mouse. *Toxicol Sci.* 116, 433-440.

Bata, A., Harrach, B., Ujszaszi, K., Kis-Tamas, A., Lasztity, R., 1985. *Macrocyclic trichothecene* toxins produced by *Stachybotrys atra* strains isolated in Middle Europe. *Appl Environ Microbiol.* 49, 678-681.

Betancourt, D.A., Krebs, K., Moore, S.A., Martin, S.M. 2013. Microbial volatile organic compound emissions from *Stachybotrys chartarum* growing on gypsum wallboard and ceiling tile. *BMC Microbiol.* 13:283. doi: 10.1186/1471-2180-13-283.

Carey, S. A., Plopper, C. G., Hyde, D. M., Islam, Z., Pestka, J. J., Harkema, J. R., 2012. Satratoxin-G from the black mold *Stachybotrys chartarum* induces rhinitis and apoptosis of olfactory sensory neurons in the nasal airways of rhesus monkeys. *Toxicol Pathol.* 40, 887-898.

Chung, Y. J., Jarvis, B., Pestka, J., 2003. Modulation of lipopolysaccharide-induced proinflammatory cytokine production by satratoxins and other macrocyclic trichothecenes in the murine macrophage. *J Toxicol Environ Health A.* 66, 379-391.

EFSA (European Food Safety Authority), 2008. Scientific information on mycotoxins and natural plant toxicants. CFP/EFSA/CONTAM/2008/01. European Food Safety Authority

EPA (U.S. Environmental Protection Agency), 2004. Guidance for clinicians on the recognition and management of health effects related to mold exposure and moisture indoors. United States Environmental Protection Agency.

Johanning, E., Biagini, R., Hull, D., Morey, P., Jarvis, B., Landsbergis, P., 1996. Health and immunology study following exposure to toxigenic fungi (*Stachybotrys chartarum*) in a water-damaged office environment. *Int Arch Occup Environ Health.* 68, 207-218.

- Kuhn, D. M., Ghannoum, M. A., 2003. Indoor mold, toxigenic fungi, and *Stachybotrys chartarum*: infectious disease perspective. *Clin Microbiol Rev.* 16, 144-172.
- McCormick, S. P., Stanley, A. M., Stover, N. A., Alexander, N. J., 2011. Trichothecenes: From simple to complex mycotoxins. *Toxins (Basel).* 3, 802-814.
- Pestka, J. J., Yike, I., Dearborn, D. G., Ward, M. D., Harkema, J. R., 2008. *Stachybotrys chartarum*, trichothecene mycotoxins, and damp building-related illness: new insights into a public health enigma. *Toxicol Sci.* 104, 4-26.
- Sorsa, M., Linnainmaa, K., Penttilä, M., Ilus, T., 1980. Evaluation of the mutagenicity of epoxytrichothecene mycotoxins in *Drosophila melanogaster*. *Hereditas.* 92, 163-145.
- Terr, A. I., 2001. *Stachybotrys*: relevance to human disease. *Ann Allergy Asthma Immunol.* 87, 57-63.
- Wannemacher, R. W., Wiener, S. L., 1997. Trichothecene Mycotoxins. In: Zajtchuk, R. (Ed.), *Medical Aspects of Chemical and Biological Warfare*. Office of The Surgeon General, Department of the Army, United States of America, Falls Church, Virginia.
- WHO (World Health Organization), 1990. Selected Mycotoxins: Ochratoxins, Trichothecenes, Ergot. *Environmental Health Criteria* 105. International Programme on Chemical Safety, World Health Organization. Available at:
<http://www.inchem.org/documents/ehc/ehc/ehc105.htm#SectionNumber:2.3>
- Yike, I., Rand, T. G., Dearborn, D. G., 2005. Acute inflammatory responses to *Stachybotrys chartarum* in the lungs of infant rats: time course and possible mechanisms. *Toxicol Sci.* 84, 408-417.