Sterigmatocystin in foodstuffs and feed: aspects to consider

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1. Background

Food and feed can be contaminated with a mixture of mycotoxins, toxic secondary metabolites produced by fungal species. Fungal invasion and consequent mycotoxin production occurs both in the field and/or during the storage of crops (de Nijs et al. 2016). The level of contamination is dependent on many factors such as fungal interactions, type of crops and environmental factors (Battilani et al. 2012; Van der Fels-Klerx 2016). In food, other aspects can influence the profile and levels of the mycotoxins present, namely, industrial processes involved and, after being acquired, household preparation process (Nijs et al. 2016). In addition, we should consider that mycotoxins are extremely difficult to eliminate from food even after the cooking process because they are quite stable molecules. All this explains why mycotoxins can be present in the food or environment long after death and disintegration of the toxic fungus (Peraica et al. 1999; Halstensen 2008; Alborch et al. 2011; Viegas et al. 2015).

2. Aspergillus section Versicolores

The identification of aspergilli down to the species level was traditionally based on the morphological features (Raper and Fennel 1965). More recently, Houbraken et al. (2014) and Hubka et al. (2014) proposed the system comprising four subgenera (Aspergillus, Circumdati, Fumigati, Nidulantes) with 20 sections based on phylogenetic approach. The official fungal DNA barcode applying ITS locus (Schoch et al. 2012) has seemed to be insufficient for accurate identification of aspergilli and their sexual morphs, so, additional marker analyses were developed: calmodulin gene (CaM), beta-tubuline (BenA) and RNA polymerase II second largest subunit (RPB2) sequencing, along with extrolite spectrum data as well (Samson et al. 2014; Frisvad 2015). Nowadays, 17 species are assigned to the Aspergillus section Versicolores: A. amoenum, A. austroafricanus, A. creber, A. cvjetkovicii, A. fructus, A. griseoaurantiacus, A. hongkongensis, A. Jensenii, A. pepii, A. protuberus, A. puulaauensis, A. subversicolor, A. sydowii, A. tabacinus, A. tennesseensis, A. venenatus and A. versicolor (Jakišić Despot et al. 2017). According to the works by Chen et al. (2016) and Hubka et al. (2016), the section Versicolores was merged to the section Nidulantes as the A. versicolor clade, to maintain monophyly of Aspergillus. The newest combined phylogeny of aspergilli at the section Versicolores based on GenBank sequences was published by Jakišić Despot et al. (2017).

Along to the typical aspergillum – a biseriate head on a long stipe, several A. versicolor and A. sydowii isolates produce diminutive conidial heads resembling
penicillate conidiophores. The recommended sexual name *Emericella* should be used for species in the section *Versicolores*. *Aspergillus versicolor* (Vuill.) Tieb. was historically the most commonly reported representative of the entire section. Its herbarium strain CBS 538.65 bears ITS barcode EF652442, *BenA* = EF652266, *CaM* = EF652354, *RPB2* = EF652178, followed by *A. sydowii* (Bainier and Sartory) Thom and Church, herb.: IMI 211,384, ITS barcode: EF652450, *BenA* = EF652302, *CaM* = EF652390, *RPB2* = EF652214 (Samson et al. 2014). So, that is why *A. versicolor* strains were reported growing worldwide, prospering in many habitats, including foodstuffs and feedstuffs as well as indoor environments, and being implicated in various human and animal health hazards, from mycoses to mycotoxoses due to production of sterigmatocystin (STC). Of note, it has been already reported that their high prevalence in some specific occupational environments is a consequence of this evidence, such as swineries (Sabino et al. 2012; Viegas et al. 2013).

An extensive search was performed to identify scientific papers, available in different scientific databases and published after 2010, reporting *Aspergillus* section *Versicolores* presence in foodstuffs and feed samples. These species have been reported in several foodstuffs such as cocoa (Egbuta et al. 2014), maize (Makun et al. 2010), date fruits (Al-Bulushi et al. 2017), dry-cured meat products (Sonjak et al. 2011), coffee beans (Batista et al. 2003; Viegas et al. 2017), wheat (Tančinová and Labuda 2009; Al-Hazmi 2010), wheat and wheat products (Riba et al. 2008; Piotrowska 2013), rice (Lima et al. 2000; Aydin et al. 2011), cereals (Tabuc et al. 2010; Toffa et al. 2013), peanut (Toffa et al. 2013), ready-to-use vegetable salads (Kocić-Tanackov et al. 2010), vegetables (Accensi et al. 2004), honey (Kačânová et al. 2012), apples with rotting (Tančinová et al. 2013), puerh tea (Haas et al. 2013), frozen chicken (Darwish et al. 2016), dried raisins (Alghalibi and Shater 2004) and spice Kashmiri chilli mild (Hammami et al. 2014). Several different feeds were reported to be contaminated, namely, maize grain (Chemining’wa et al. 2009; Ayalew 2010), fish feed (Barbosa et al. 2013), feed mixture (Magnoli et al. 2002; Accensi et al. 2004; Labuda and Tančinová 2006), silage (El-Shanawany et al. 2005), soybean (Kačânov 2003), cereals (Accensi et al. 2004; Tabuc et al. 2009, 2011), raw materials like corn and barley rootlets (Rosa et al. 2008), soybean meals (Al-Seeni 2012) and sorghum (Silva et al. 2000) (Table 1).

### 3. STC production ability

Mycotoxin STC synthesis is restricted to species in four sections in *Aspergillus* (*Ochraceorosei*, *Versicolores*, *Nidulantes* and *Flavi*) (Rank et al. 2011). Most of the aspergillus species from the section *Versicolores* are able to produce STC, namely, *A. amoenus*, *A. creber*, *A. cvjetkovicii*, *A. fructus*, *A. griseouranticus*, *A. hongkongensis*, *A. jenseni*, *A. pepii*, *A. protuberus*, *A. pululauensis*, *A. subversicolor*, *A. tennesensis*, *A. venenatus* and *A. versicolor* (Jurjevic et al. 2013; Visagie et al. 2014; Jakšić Despot et al. 2017). According to Frisvad (2015), the metabolic profile is unique for each fungal species entity with a high degree of chemoconsistency among different isolates of the particular species. But, so far, at the section *Versicolores*, there are chemical markers characterised just for *A. versicolor* and *A. sydowii* (Samson et al. 2010). Liquid Chromatography Mass Spectrometry (LC-MS) LC/MS-based methods proved to be accurate to identify chromatograms of fungal extracts in general. At the moment, some extra-tolites of *A. versicolor* were found, like polyketides (Lee et al. 2010), stephacidin A and notoamide B (Greshock et al. 2008) or kipukasins (Jiao et al. 2007), respectively.

STC and dihydrosterigmatocystin are the penultimate precursors of aflatoxins – polyketide-derived furanocoumarins. It has been demonstrated that 25 identified genes clustered within a 70-kb DNA region in the chromosome are involved in their biosynthesis (Townsend 1997). The homologous genes and their corresponding enzymes acting in each bioconversion step in the biochemical pathway common to aflatoxins and STC were described later on as well (Yu et al. 2004). For example, in *A. nidulans*, the last in the row crucial gene seems to be *stcP* encoding O-methyltransferase B required for the conversion of dimethylsterigmatocystin to STC (Kelkar et al. 1996).

### 4. STC toxicity

STC is a possible human carcinogen (2B) according to IARC classification (McConnell and Garner 1994) and showed immunotoxic and immunomodulatory activity (Liu et al. 2014), together with mutagenic effects (Gao et al. 2015). It might be found in numerous
| Setting                                                                  | Food or feed sampled                  | Number of samples | Analytical methods                                                                 | Results                                      | Reference                      |
|------------------------------------------------------------------------|---------------------------------------|-------------------|-------------------------------------------------------------------------------------|---------------------------------------------|--------------------------------|
| Rough rice warehouse from Rio Grande do Sul Brazil                     | Polished rice                         | 30                | Plated onto Potato dextrose agar; plates were incubated for 7 days at 25°C          | Prevalence 6.6%                            | Lima et al. (2000)              |
| Freshly harvested and stored sorghum from the State of São Paulo, Brazil| Sorghum                               | 140               | Inoculated onto potato dextrose agar; incubated at 25°C for 5 days and observed daily | 1.4% prevalence                            | Silva et al. (2000)             |
| Mixed feed production plant from Cordoba, Argentina                    | Poultry feed                          | 120               | Inoculated onto DRBC agar; incubated in darkness at 28°C for 7 days                 | 1.0 × 10^1–5.0 × 10^3 (CFU g^-1)            | Magnoli et al. (2002)           |
| Eleven municipalities supermarkets from Brazil                          | Processed coffee beans (Coffee arabica L.) | 45                | Plated directly onto filter paper moistened with sterile distilled water; 15 were plated without prior hypochlorite disinfection and 15 were disinfected with 1% hypochlorite for 2 min | 3 isolates                                | Batista et al. (2003)           |
| Agricultural companies in the years 2002 and 2003 from Slovak Republic | Soybean                               | 25                | Inoculated onto Czapek-Dox agar with streptomycin; incubated at 25°C 10 days        | 2 isolates                                  | Kacaníova (2003)                |
| Local factories                                                        | Mixed feeds                           | 147               | Inoculated onto malt extract agar; incubated at 28°C for 7 days                     | Prevalence 9.5%                             | Accensi et al. (2004)           |
| Dried fruits from shops and markets in Yemen Republic                  | Cereals                               | 117               | Inoculated at 28°C for 10 days                                                     | Prevalence 14.5%                            |                              |
| Dried raisins, figs and dates                                          | Vegetables                            | 36                | Inoculated at 28°C for 10 days                                                     | Prevalence 8.3%                             |                              |
| Farms, mills and retail markets at Assuit and Sohag governorates from Egypt | Silage                               | 40                | Inoculated onto abouraud's dextrose agar with chloramphenicol and cyclohexemide; -incubated at 28°C for 1–2 weeks | 3 isolates                                  | Alghalibi and Shater (2004)     |
| Poultry farms from Slovakia                                           | Feed mixture                          | 108               | Inoculated onto Dichloran chloramphenicol peptone agar (DCPA); incubated at 25°C for 5–7 days; conidial suspensions inoculated onto Czapek Yeast Extract Agar (CYA). Czapek Yeast Extract Agar CY20S and Malt extract agar (MEA), and incubated in the dark at 25°C for 7–14 days | Occurrence in 13 samples with 33 isolates   | Labuda and Tančinová (2009)    |
|                     | Wheat and wheat products              | 30                | Inoculated onto Dichloran Rose Bengal Chloramphenicol Agar (DRBC) Agar; incubated for 5–7 days at 28°C in the dark; colonies sub-cultured on malt extract agar; allowed to grow at 28°C for 7–10 days | Frequency ranged from 10% to 28%           | Riba et al. (2008)              |
|                      | Flour mill                            | 12                | Inoculated onto Dichloran Rose Bengal Chloramphenicol Agar (DRBC) Agar; incubated for 5–7 days at 28°C in the dark; colonies sub-cultured on malt extract agar; allowed to grow at 28°C for 7–10 days |                              |                              |
|                      | Semolina mill and samples from Algeria region | 12              | Inoculated onto Dichloran Rose Bengal Chloramphenicol Agar (DRBC) Agar; incubated for 5–7 days at 28°C in the dark; colonies sub-cultured on malt extract agar; allowed to grow at 28°C for 7–10 days |                              |                              |
|                      | Cattle dairy feed from Rio de Janeiro State, Brazil |                  | Inoculated onto DRBC; incubated at 28°C for 7 days | Corn prevalence11.8% and barley rootlets prevalence 8.1% | Rosa et al. (2008) |
|                      | Raw material (corn, brewe's grain, barley rootlets, cotton flour, pelleted citric pulp) |              | Inoculated onto DRBC; incubated at 28°C for 7 days | Corn prevalence11.8% and barley rootlets prevalence 8.1% | Rosa et al. (2008) |
|                      | Finished cow feed                     | 24                | Inoculated onto Czapek dox agar; incubated at 25°C for 7–10 days                     | Positive results in 6 zones, percentage ranging from 0.6% to 6.6% | Chemining'wa et al. (2009)     |
| Nine agro-ecological zones from Eastern Kenya                          | Maize grain                           | 5 farmers per ecological zone | Inoculated onto Czapek dox agar; incubated at 25°C for 7–10 days                     | Positive results in 6 zones, percentage ranging from 0.6% to 6.6% | Chemining'wa et al. (2009)     |

(Continued)
Table 1. (Continued).

| Setting                                      | Food or feed sampled          | Number of samples | Analytical methods                                                                 | Results                              | Reference                             |
|----------------------------------------------|-------------------------------|-------------------|------------------------------------------------------------------------------------|--------------------------------------|---------------------------------------|
| Cereal samples from Southeastern Romania     | Corn                           | 54                | Plated onto malt agar and malt salt agar; colonies were counted after 3, 5, and 7 days of culture at 25°C and 31°C | Prevalence of 46.2%                  | Tabuc et al. (2009)                    |
|                                              | Wheat                         | 35                |                                                                                    | Prevalence of 22.8%                  |                                       |
|                                              | Barley                        | 21                |                                                                                    | Prevalence of 9.4%                   |                                       |
| Mills from Slovakia                          | Wheat bran                    | 56                | Inoculated onto Dichloran chloramphenicol pepton agar (DCPA); 5–7 days of incubation at 25°C | Occurrence in 5 samples with 8 isolates | Tančinová and Labuda (2009)           |
|                                              | Wheat grain                   | 30                | Inoculated onto potato dextrose agar (PDA); incubated at 25°C in dark for 7 days     | 2 × 10³ colony/g                     | Al-Hazmi (2010)                       |
| Wheat from different production areas and sold Jeddah market, Saudi Arabia | Maize grain                  | 17                | Inoculated onto Dichloran Glycerol (DG18 agar; incubated at room temperature for 1–2 weeks | Surface prevalence 23.9%             | Ayalew (2010)                         |
| 2004/2005 harvest in Ethiopia                | Salads                        | 17                | Inoculated to DRBC agar; incubated at 25°C for 5–7 days                          | Prevalence of 1.78%                 | Kocić-Tanackov et al. (2010)          |
| Fresh ready-to-use salads made of different types of vegetables in Serbia | Five different food commodities | 343              | Inoculated onto PDA; incubated 5–7 days at 28°C                                  | 2 isolates                          | Makun et al. (2010)                   |
| Maize field, market and storage facilities from three States in Nigeria | Cereal grain (corn, wheat, barley, oats) | 56 | Applied onto malt and agar with 6% NaCl; incubated for 5–7 days at 25°C          | 10.7% prevalence                    | Tabuc et al. (2010)                   |
| Cereal samples from different locations in Banat region, Romania | Rice                          | 100               | Inoculated onto DRBC agar; incubated at 25°C for 5–7 days; Colonies sub-cultured on malt extract agar incubated at 25°C for 5–7 days | 2 positive samples                  | Aydin et al. (2011)                   |
| Two rice-growing areas in the Thrace region from Turkey |                     |                   |                                                                                    |                                      |                                       |
| Slovenian meat-processing plant              | Dry-cured meat products       | 75 items of different dry-cured meat products | Inoculated to MEA and to MEA with 5% NaCl; isolates inoculated onto MEA and grown for 7 days at 25°C in the dark | 2 isolates                          | Sonjak et al. (2011)                  |
| Cereals produced in the South East part of Romania between 2008 and 2010 | Feed cereals (maize, wheat, barley, oats, rye, soya, sunflower, colza, rice, triticale) | 86 | Plated onto malt agar medium and malt salt agar medium; incubated 7 days at 25°C and 31°C | 2008 (n = 42) 38.0% 2009 (n = 32) 31.2% 2010 (n = 12) 58.3% | Tabuc et al. (2011)                   |
| Soybean meal collected from fodder markets at Jeddah, Saudi Arabia | Soybean meals                 | 20                | Inoculated onto Czapek-Dox agar with 1% streptomycin; incubated at 25°C 10 days       | 1.4 × 10⁸ count/g                    | Al-Seenin (2012)                      |
| Bee-keepers (apairy honey) from Slovakia and other countries | Honey                         | 53                | The cultures grew under specific conditions on Czapek-Dox agar and Malt agar         | 3 positive samples with frequency 6.82% | Kačániová et al. (2012)               |
| Two tilapia farmsins the Rio de Janeiro State, Brazil | Fish feed                     | 60                | Inoculated onto DRBC and DG18; incubated at 25°C for 5–7 days                    | 16% relative density                | Barbosa et al. (2013)                 |
| Different stores and markets in south-western Nigeria | Processed cocoa               | 22                | Inoculated onto PDA, OAES Aohio agricultural experimental station agar, MEA and CYA; incubated 4–7 days at 25°C | 2.0 × 10⁴ (Cfu/g) 4.6%              | Egbuta et al. (2013)                  |
| Setting | Food or feed sampled | Number of samples | Analytical methods | Results | Reference |
|---------|----------------------|-------------------|--------------------|---------|-----------|
| Tea shops from the province of Yunnan in southwestern China | Pu-erh tea | 36 | Plated onto MEA, Sabouraud Glucose Agar and DG18; Incubated at 25°C 7–14 days | 2.8% prevalence | Haas et al. (2013) |
| Cereal breakfast products from Lodz (Poland) markets | Cereal snacks with cinnamon, cornflakes, cornflakes with nuts and honey, multi-cereal products, cereal products with chocolate and muesli with dried fruit, nuts, cereal and coconut flakes | 15 | Inoculated onto DRBC agar and DG18; incubated at 25°C for 7 days | Cereal snacks with cinnamon – 37%; cornflakes with nuts and honey – 24%; multi-cereal products – 19%; muesli – 20%; cereal products with chocolate – 50% and 10% | Plotrowska (2013) |
| Apples with the characters of rotting bought in the commercial network from Slovakia | Rotting apples | 30 | Roducers of rotting were inserted directly onto MEA Cultivated 5–7 days in the dark at 25 ± 1 °C; Aspergillus sp. isolated to CYA, MEA, CY20S, CREA creatine sucrose agar and YES; cultivation proceeded for 5–7 days in the dark at 25 ± 1°C | 1 isolate | Tančinová et al. (2013) |
| Cereals and peanut from local markets from Republic of Niger | Cereals (rice, maize, sorghum, millet) and peanut | 81 | Plated onto PDA, CYA, MEA; Incubated MEA 30°C, PDA 27°C, CYA 25°C for 7 days | 4 strains | Toffa et al. (2013) |
| Powdered spices from Qatar local markets | Spices: chili, Kashmiri chili hot, Kashmiri chili mild, basil, oregano, ginger, curry, cumin, turmeric, tandoori masala, garam masala, black pepper, garlic and coriander | 14 | Inoculated onto DRBC agar; Incubated at 28°C 5 days | In Kashmiri chili mild 3 isolates | Hammami et al. (2014) |
| Frozen chicken from different localities in Zagazig city, Egypt | Breast | 20 | Culturing on malt extract agar media and Czapeck-Dox agar with 5% NaCl; incubation in dark at 25°C for 5–7 days | 10% | Darwish et al. (2016) |
| | Thigh | 20 | | 10% | |
| | Gizzards | 20 | | 14–15% | |
| | Livers | 20 | | 4–5% | |
| Several date palm from Oman region | Date palm (Tamar stored dates) | 3 date fruits from each cultivar. | Illumina MiSeq Sequencing Analysis | Frequency of occurrence in Khenizi mesocarp 0.32 and skin 0.065 | Al-Bulushi et al. (2017) |
| Green coffee beans from Coffea arabica (Arabica coffee) and Coffea canephora var. robusta (Robusta coffee) to be roasted in Portugal | Samples from different countries of origin | 28 | The washed supernatant (100 µl) was plated in MEA and DG18; incubated at 27°C for 5 to 7 days. | 6.1 prevalence | Viegas et al. (2017) |
substrates, from foods and feeds to chronically damp building materials and indoor dust.

In 2013, the European Food Safety Authority (EFSA) was asked by the European Commission to deliver a scientific opinion on STC in food and feed. The Panel on Contaminants in the Food Chain (CONTAM) from EFSA was responsible for this opinion. However, due to the absence of exposure data for the European population, the margin of exposure approach for substances that are genotoxic and carcinogenic could not be applied for STC, and therefore, the risk of STC for human health was not characterised.

Despite this, it was possible to collect all the available information related to STC toxicokinetics, toxicity, mode of action and dose-response assessment by comparing with aflatoxin B1. The following information was available in the EFSA Journal and, more precisely, in the scientific opinion on the risk for public and animal health related to the presence of STC in food and feed (EFSA Panel on Contaminants in the Food Chain (CONTAM) 2013).

This report concludes the exposure to STC to be of low concern for public health based on the relative carcinogenic potency of STC and AFB1 and exposure data. However, the need of data concerning exposure of European citizens was also mentioned (EFSA Panel on Contaminants in the Food Chain (CONTAM) 2013).

4.1. STC toxicokinetics

There is limited information available related to STC toxicokinetics. However, the accessible data suggests that absorption of STC is limited following oral exposure.

In the same way, data on the biotransformation of STC is also insufficient. Few studies published to date indicate that phase I metabolism of STC comprises cytochrome P450 (CYP450)-mediated formation of a reactive epoxide as well as monohydroxylation and dihydroxylation reactions. In a more detailed manner, STC is metabolised in the liver and lung by various CYP450 enzymes into different hydroxymetabolites and its reactive exo-epoxide that readily forms DNA adducts (EFSA Panel on Contaminants in the Food Chain (CONTAM) 2013; Walkow et al. 1985).

As phase II metabolites, a glucuronide of STC and of monohydroxy-STC has been observed and reported, together with a sulphate conjugate of monohydroxy-STC and a glutathione adduct of a monooxygenated STC. Excretion of both conjugated parent STC and its hydroxylated metabolites occurs via bile and urine. Nevertheless, the structure of most of these metabolites is not completely known and more research is necessary to allow the availability of more detailed information (Walkow et al. 1985; EFSA Panel on Contaminants in the Food Chain (CONTAM) 2013).

4.2. Toxicity of STC

Due to the structural similarities, aflatoxins and STC share relevant toxic effects, including genotoxicity and carcinogenicity (Miller and Trenholm 1994; EFSA Panel on Contaminants in the Food Chain (CONTAM) 2013). However, in contrast to aflatoxins, only limited information on occurrence and toxicity of STC is available.

Liver and kidneys are the target organs of acute toxicity. However, the acute oral toxicity is relatively low (range between 120 and 166 mg/kg body weight). STC is hepatotoxic in rat, mouse, monkey and guinea pig. The incidence of hepatocellular necrosis and haemorrhages increases with dose and duration of exposure. In the kidney, hyaline degeneration, tubular necrosis and haemorrhages were described in rats and/or monkeys exposed to STC (Purchase and Van Der Watt 1969; EFSA Panel on Contaminants in the Food Chain (CONTAM) 2013).

Results from in vivo and in vitro studies suggest that STC may have also immunomodulatory activity, but strong conclusions cannot be drawn (Huang et al. (2002), Xing et al. (2005), and Zhang et al. (2012) cited in EFSA Panel on Contaminants in the Food Chain (CONTAM) 2013).

STC is mutagenic in both bacterial and mammalian cell assays after metabolic activation. Subsequently, STC induces chromosomal damage both in vitro and in vivo in experimental animals (Curry et al. 1984; Ueda et al. 1984; Mori et al. 1986; Crofton-Sleigh et al. 1993; Abdel-Wahhab et al. 2005). Various studies aimed to compare the genotoxicity of STC and AFB1. However, the uncertainty regarding their actual concentration in the test system, the efficiency of the activation/detoxification metabolic routes and the repair rate of induced lesions does not allow a direct comparison of the
relative mutagenic potency of these mycotoxins (EFSA Panel on Contaminants in the Food Chain (CONTAM) 2013).

In previous studies, tumourigenicity of STC was observed after oral, intraperitoneal, subcutaneous and/or dermal administration in the animal species tested (rat, mouse, Mongolian gerbils, monkey and fish). After oral exposure, premalignant and malignant lesions such as hepatocellular carcinomas (HCC), haemangiosarcomas in the liver, angiosarcomas in the brown fat, lung adenomas and incidental findings in other organs were reported (EFSA Panel on Contaminants in the Food Chain (CONTAM) 2013).

Based on the available information, the CONTAM Panel of EFSA concluded that STC is genotoxic and carcinogenic (EFSA Panel on Contaminants in the Food Chain (CONTAM) 2013).

Additionally, a study developed by Miller et al. (2010), which considered exposure of STC via the indoor environment, concluded that following inhalation (intratracheal installation) of STC, a non-specific but severe inflammatory response of the lung tissue was observed. Similarly, severe cytotoxic and inflammatory damage of lung tissue as well as breaking down of self-cleaning mechanism of airways in rats in vivo were observed after intratracheal instillation of STC containing complex extrolites of an A. versicolor strain of indoor origin in the studies by Piecková et al. (2011, 2015).

4.3. STC mode of action

The mode of action of STC can be described as follows. Phase I metabolism results in metabolic activation that promotes the formation of N7-guanyl DNA adducts. These adducts are likely to be responsible for the STE mutagenic effects (Essigmann et al. 1979, 1980). A dose-dependent formation of DNA adducts of STC was found in the concentration range between 1 and 3 mg STC per liver (Reddy et al. 1985; cited in EFSA Panel on Contaminants in the Food Chain (CONTAM) 2013).

STC induces cytotoxicity, inhibition of cell cycle and mitosis, as well as an increased formation of reactive oxygen species (ROS) and lipid peroxidation in vivo (Kawai et al. 1984; Ueno et al. 1995; Xie et al. 2000; Sivakumar et al. 2001; Bünger et al. 2004).

The conclusion made by EFSA Panel on Contaminants in the Food Chain (CONTAM) (2013) was that the genotoxicity of STC is based on the formation of DNA adducts that, if unrepaired, increase the likelihood of mutation fixation. Moreover, when comparing with AFB1, most in vitro studies with purified DNA indicate that the level of induced N7-guanyl adducts is higher after AFB1 than STC exposure, supporting the view that AFB1 is a more potent liver carcinogen than STC. Various in vitro and in vivo investigations have demonstrated that STC exerts cytotoxicity, inhibition of cell cycle and mitosis, as well as an increased ROS formation and lipid peroxidation in vivo. However, most of the in vitro assays have been conducted with rather high STC concentrations, not representing the real human exposure scenario that should be a chronic exposure. Therefore, the observed effects of those studies have to be interpreted with caution not allowing to make conclusions regarding the potential adverse effects of (low dose) dietary exposure to STC (EFSA Panel on Contaminants in the Food Chain (CONTAM) 2013).

More recently, a study developed by Wang et al. (2015) tried to confirm that STC exposure is a risk factor for oesophageal cancer and that STC may induce DNA damage and G2 phase arrest in immortalised human oesophageal epithelial cells (Het-1A). Indeed, the study developed allowed to conclude that STC can induce different cell cycle arrest in primary human oesophageal epithelial cells and immortalised human oesophageal epithelial cells in vitro (Wang et al. 2015).

In 2017, Jiang and co-authors aimed to investigate whether checkpoint adaptation occurs in GES-1 Cellosaurus cell line (GES-1) cells treated with STC. The results suggested that STC induces an initial G2 arrest that is subsequently followed by G2 phase checkpoint adaptation, which may potentially promote genomic instability and result in tumorigenesis (Jiang et al. 2017).

Additionally, a study developed by Huang et al. (2014) in human pulmonary cells in vitro observed that STC induced DNA damage and affected key proteins involved in cell cycle regulation to trigger genomic instability, which may be a potential mechanism underlying the developmental basis of lung carcinogenesis.

4.4. Dose–response modelling

Despite the evidence on genotoxicity and carcinogenicity, only a limited tumourigenicity database was
available for dose–response assessment since most of the studies published have several limitations that do not allow to be used for dose–response modelling (EFSA Panel on Contaminants in the Food Chain (CONTAM) 2013).

Being aware of this limitation, the CONTAM Panel of EFSA compared the carcinogenic potency of STC and AFB1 in the BMD10 values. After the comparison of the BMD10 of STC for the occurrence of haemangiosarcomas and that of AFB1 for the occurrence of HCC, the CONTAM Panel concluded the carcinogenic potency of STC is approximately three orders of magnitude lower than that of AFB1 (EFSA Panel on Contaminants in the Food Chain (CONTAM) 2013).

5. Food and feed contamination already reported

The natural occurrence of STC in foodstuffs and feed has been reported in a limited number of surveys (Table 2). Reports from year 2000 to 2017 have presented STC contamination in several foodstuffs such as rice (Sawane and Sawane 2014; Mo et al. 2015; Rofiat et al. 2015; Bertuzzi et al. 2017), bread (Versílovskis and Bartkevičs 2012), wheat bran (Tančinová and Labuda 2009), grain (Versílovskis et al. 2008a; Mo et al. 2015), maize (Warth et al. 2012; Mo et al. 2015), groundnuts (Warth et al. 2012), peanut seed (Youssef et al. 2008), coffee bean (Bokhari and Aly 2009; Culliao and Barcelo 2015), beer (Versílovskis et al. 2008b), cheese (Versílovskis et al. 2009), cereal grains, cereal products (Mo et al. 2015) and almond seed (Yassin et al. 2013). Additionally, some reports in animal feed found contamination in grass (Nichea et al. 2015), feed mixture (Labuda and Tančinová 2006; Warth et al. 2012), wheat, corn, barley, soybean, sunflower cake (Kovalenko et al. 2011), silage (El-Shanawany et al. 2005), straw and hay (Mol et al. 2014).

Liquid chromatography-tandem mass spectrometry was used for STC detection in nine studies with limits of detection (LOD) ranging from 0.03 to 2.0 µg/kg and five out of nine had also limits of quantification (LOQ) ranging from 0.1 to 0.5 µg/kg (Warth et al. 2012; Versílovskis et al. 2008a; b, 2009; Versílovskis and Bartkevičs 2012; Mol et al. 2014; Rofiat et al. 2015; Nichea et al. 2015; Bertuzzi et al. 2017). Also thin-layer chromatography (El-Shanawany et al. 2005; Labuda and Tančinová 2006; Youssef et al. 2008; Bokhari and Aly 2009; Tančinová and Labuda 2009; Sawane and Sawane 2014; Culliao and Barcelo 2015), high-performance liquid chromatography (Versílovskis et al. 2008a; b; Yassin et al. 2013; Culliao and Barcelo 2015) and enzyme-linked immunoassay (Kovalenko et al. 2011) analytical methods were used to determine STC in samples. However, in some of these studies, LOD or LOQ values were not available.

6. Climate change influence on STC production

Climate change has been occurring since the earth existed, and global temperatures normally show that 7 of the top 10 warmest years on record have occurred since the 1990s. The decade of 2000–2009 was the warmest period worldwide (EPA 2010). By 2100, the atmospheric concentration of CO₂ is predicted to rise up to the range of 540 and 970 ppm above the current concentration. Together with other greenhouse gases such as CH₄, this will lead to a predicted global temperature increase of 1.1–6.4°C, depending on different models used and global region (In et al. 2007; Battilani et al. 2012). There is increased risk of European countries with temperate climates to have the higher exposure to fungi and mycotoxins due to climate change, which has already been identified by some authors (Paterson and Lima 2011; Battilani et al. 2012). The climate of these countries will probably become warmer reaching temperatures of 33°C, which is, for instance, a temperature very close to the optimal temperature for Aspergillus section Versicolores growth (30°C) (Atalla et al. 2003) and STC production (optimal temperature between 23°C and 29°C) (Atalla et al. 2003). However, effects of climate change on fungal species distribution and activity are difficult to predict because they are influenced in many different ways such as fungal characteristics, host features and availability, and competitive interactions between microbiota. In addition, environmental variables such as temperature, water availability and atmospheric CO₂ and the interaction of these variables make it difficult to predict their influence on fungal distribution (Boddy 1984) and, consequently, mycotoxin presence in food and feed even higher (In et al. 2007). Mycotoxins are profoundly dependent on climate, plant and storage-associated problems, and also influenced by non-infectious factors (e.g.
Table 2. Sterigmatocystin prevalence in foodstuffs and feed samples.

| Setting                                    | Food and feed sampled | Number of samples | Extraction method | Analytical method               | Results (quantitative and qualitative) | Reference                  |
|--------------------------------------------|-----------------------|-------------------|-------------------|---------------------------------|----------------------------------------|----------------------------|
| Assuit and Sohag farms, mills and retail   | Silage                | 40                | Chloroform extract| TLC                             | 2 samples                              | El-Shanawany et al. (2005) |
| markets                                    |                       |                   |                   |                                 |                                        | Labuda and Tančinová (2006) |
| Poultry farms                              | Feed mixture          | 108 samples, 5132 isolates | chloroform:methanol (2:1, v/v) | TLC                             | 10 isolates                           | Veršilovskis et al. (2008a) |
| Different parts of Latvia                  | Grain samples         | 1. In 2006 95 samples. 2. In 2007 120 samples | 84% acetonitrile in water | -LC–MS/MS analysis -An electrospray ionization (ESI) | 1. 13.7% (13 samples) from 2006 (0.7 to 83 µg/kg) 2. 35% (42 samples) from 2007 (1 to 47 µg/kg) | Veršilovskis et al. (2009) |
| Latvia local supermarkets                  | Beer (9 dark, 17 light) | 26                | Acetonitrile:water (10:90, v/v) | HPLC-UV analysis                | In 2 samples: 4.0 µg/l (light beer), 7.8 µg/l (dark beer) | Veršilovskis et al. (2008b) |
| Peanut seed                                | Fruit=20 Roasted=20   | 60                | Cyclohexane for 10 h using Soxlet type extractor | TLC                             | Roasted seed: 3 samples 16.8 µg/kg, 12.2 µg/kg, 14.8 µg/kg Roasted with salt seed: in 1 sample 12.2 µg/kg | Youssef et al. (2008) |
| Toyota city of Jeddah markets             | Coffee bean seed      | 30                | Chloroform and purified by phenyl-bond solid phase | TLC                             | In 3 samples: 13 ng/g, 11 ng/g, 5 ng/g | Bokhari and Aly (2009) |
| Mills                                      | Wheat bran            | 8 isolates from 5 samples | Chloroform:methanol 2:1 v/v | TLC                             | All 7 isolates                         | Tančinová and Labuda (2009) |
| Latvia and Belgium local supermarkets      | Cheese                             | Latvia = 8 Belgium = 13 | Acetonitrile–water (90 : 10, v/v) and n-hexane | LC-MS/MS system                   | Latvia – in 4 samples 2 × 0.04, 0.07, 0.03 (µg/kg). Belgium – in 3 samples 1.23, 0.03, 0.52 (µg/kg). | Veršilovskis et al. (2009) |
| Pig farms 2006–2009                        | Several samples from different materials | Wheat – 93 Corn – 111 Barley – 146 Soybean – 15 Sunflower cake – 53 Mixed feed for piglets of group 0–2 months/2–4 months, sows and fattening – 120/94 | ——— | ELISA method | 2008: 8% of corn samples; 2006:7–17% of wheat, soybean, pea, and mixed feeds for piglets of age groups 0–2 and 2–4 months; in 2007, 9–22% of samples of wheat, corn, barley, pea, bran, sunflower cake, and mixed feeds for piglets of age group 0–2 months and sows; in 2009, 8–50% of samples of all feed types, except mixed feeds for piglets of age group 2–4 months and sows | Kovalenko et al. (2011) |
| Foods and feedstuffs from Burkina Faso     | Maize = 26, feed = 4 and others = 30 | Total 122 | Acetonitrile/water/acetic acid (79:20:1, w/v/v) | LC-MS/MS analysis | Maize: 2 samples, median 2.3 µg/kg Feed: 3, median 6.5 µg/kg Others 2 median 6.7 µg/kg | Warth et al. (2012) |
| Foods and feedstuffs from Mozambique       | Maize = 13, groundnuts = 23, feed = 10 and others = 7 |                       |                   |                                 |                                        |                            |
| Bread samples from Riga local supermarkets | Plain rye bread       | 6                 | 16% water in acetonitrile | LC-MS/MS and ESI               | In 1 sample = 2.4 µg/kg | Veršilovskis et al. (2012) |
|                                           | Mixed rye-wheat bread | 9                 |                   |                                  | In 1 sample = 7.1 µg/kg                |                            |
|                                           | Plain wheat bread     | 14                |                   |                                  | In 3 samples = 4.4 µg/kg; 3.2 µg/kg; 5.6 µg/kg |                            |
| Different locations in Riyadh city, Kingdom of Saudi Arabia | Almond seed | 20                | Acetone, chloroform | HPLC | 300–440 ppb | Yassin et al. (2013) |
| Setting                          | Food and feed sampled | Number of samples | Extraction method                          | Analytical method | Results (quantitative and qualitative) | Reference |
|---------------------------------|-----------------------|-------------------|--------------------------------------------|-------------------|----------------------------------------|------------|
| India rice                      | Rice                  | 36                | Chloroform/methanol (2:1, v/v), mycelia    | TLC               | 5 isolates out of 12                    | Sawane A and Sawane M (2014) |
| Animal Feed                     | Straw                 | 149               | Acetonitrile/water (84/16) containing 1% formic acid | LC-MS/MS analysis | 2 samples 0.001–0.038 mg/kg             | Mol et al. (2014) |
| 2 beef cattle farms             | Hay                   | 43                | Acetonitrile/water/acetic acid (79:20:1, v/v/v) | LC-MS/MS System with ESI source HPLC System | 90% in 2011 and 60% in 2014 | Nichea et al. (2015) |
| 143 rice processors             | Milled rice           | 38                | Acetonitrile/water/acetic acid (79:20:1, v/v/v) | LC-MS/MS with ESI source and HPLC System | 50% of rice samples, mean value 19 µg/kg, maximum value 125 µg/kg | Rofiat et al. (2015) |
| Domestic and imported cereals and cereal products from different countries in different regions within the European Union | Cereal grains: Wheat | 221               | Acetonitrile-water 80/20 v/v | LC-MS/MS analysis | Wheat: 12 samples (9 = LOD-0.5 µg/kg, 3 = 0.5–1.5 µg/kg); rye: 2 samples (1 = LOD-0.5 µg/kg, 1 = 0.5–1.5 µg/kg); maize: 2 samples (0.5–1.5 µg/kg); Rice: 27 samples (8 = LOD-0.5 µg/kg, 8 = 0.5–1.5 µg/kg, 10 = 1.5–5 µg/kg, 1 = 5 µg/kg); barley: 1 sample (1.5–5 µg/kg); oats: 11 samples (4 = LOD-0.5 µg/kg, 5 = 0.5–1.5 µg/kg, 1 = 1.5–5 µg/kg, 1 = 5 µg/kg) | Mo et al. (2015) |
| Rice                            | 35                    |                   |                                             |                   |                                        |            |
| Maize                           | 33                    |                   |                                             |                   |                                        |            |
| Barley                          | 59                    |                   |                                             |                   |                                        |            |
| Oats                            | 51                    |                   |                                             |                   |                                        |            |
| Rice                            | 28                    |                   |                                             |                   |                                        |            |
| Barley                          | 28                    |                   |                                             |                   |                                        |            |
| Oats                            | 59                    |                   |                                             |                   |                                        |            |
| Rice                            | 28                    |                   |                                             |                   |                                        |            |
| Barley                          | 59                    |                   |                                             |                   |                                        |            |
| Oats                            | 51                    |                   |                                             |                   |                                        |            |
| Coffee beans from production    | Coffee beans          | 42                | Chloroform                                  | TLC and HPLC      | Mean concentrations 55.8, 161.7 and 193.7 µg/kg | Culliao and Barcelo (2015) |
| Farms and storage facilities    | Paddy rice            | 49                | Acetonitrile/water 80/20 v/v                | LC-MS/MS system. | All samples in the range of 0.29–15.85 µg/kg | Bertuzzi et al. (2017) |
| Retail and wholesale resources  | Processed rice        | 83                |                                             |                   | In 21% of samples (n = 19) 0.12 and 1.32 µg/kg (brown rice) |            |
bioavailability of (micro)nutrients, insect damage, and other pests attack) that are also driven by climatic conditions. Therefore, climate represents the crucial factor for agro-ecosystem powering fungal colonisation and mycotoxin production (Magan et al. 2003).

7. Future perspectives regarding food and feed contamination

The report by the CONTAM Panel from EFSA mentioned the need for more occurrence data on STC in food and feed across European countries to allow an accurate assessment of dietary exposure. Furthermore, the prediction of climate change and how it can influence fungal contamination and mycotoxin production should be considered. Therefore, besides not knowing in detail what is the actual exposure to this mycotoxin in Europe, the new scenario of climate change brings new challenges due to a probable new exposure trends, particularly in countries with temperate climate.

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References

Abdel-Wahhab MA, Hasan AM, Aly SE, Mahrous KF. 2005. Adsorption of sterigmatocystin by montmorillonite and inhibition of its genotoxicity in the Nile tilapia fish (Oreochromis niloticus). Mutat Res. 582:20–27.

Accensi F, Abarca ML, Cabañas FJ. 2004. Occurrence of Aspergillus species in mixed feeds and component raw materials and their ability to produce ochratoxin A. Food Microbiol. 21(5):623–627.

Alborch L, Bragulat MR, Abarca ML, Cabañas FJ. 2011. Effect of water activity, temperature and incubation time on growth and ochratoxin A production by Aspergillus niger and Aspergillus carbonarius on maize kernels. Int J Food Microbiol. 147:53–57.

Al-Bulushi IM, Bani-Uraba MS, Guizani NS, Al-Khudaibi MK, Al-Sadi AM. 2017. Illumina MiSeq sequencing analysis of fungal diversity in stored dates. BMC Microbiol. 17:72.

Alghalibi SMS, Shater ARM. 2004. Mycoflora and mycotoxin contamination of some dried fruits in Yemen Republic. Ass Univ Bull Environ Res. 7:2.

Al-Hazmi NA. 2010. Determination of zearalenone (ZEA) in wheat samples collected from jeddah market, Saudi Arabia. Afr J Microbiol Res. 4(23):2513–2519.

Al-Seeni MN. 2012. Natural occurrence of heavy metal, fungi and mycotoxins in soybean meal samples used in animal feeding in Saudi Arabia. Afr J Biotechnol. 11(38):9288–9294.

Atalla MM, Hassanein NM, El-Beih AA, Youssef YA. 2003. Mycotoxin production in wheat grains by different Aspergilli in relation to different relative humidities and storage periods. Nahrung. 47:6–10.

Aydin A, Aksu H, Günsen U. 2011. Mycotoxin levels and incidence of mould in Turkish rice. Environ Monit Assess. 178:271–280.

Barbosa TS, Pereyra CM, Soleiro CA, Dias E, Oliveira A, Keller KM, Silva P, Cavagliera LR, Rosa C. 2013. Mycobiota and mycotoxins present in finished fish feeds from farms in the Rio de Janeiro State, Brazil. Int Aquatic Res. 5:3.

Batista LR, Chalfoun SM, Prado G, Freitas Schwran R, E. Wheals A. 2003. Toxicogenic fungi associated with processed (green) coffee beans (Coffeea arabica L.). Int J Food Microbiol. 85(3):293–300.

Battilani P, Rossi V, Giorni P, Gualla A, Van der Fels-Klerx HJ, Booij CJH, Moretti A, Logrieco A, Toscano P, et al. 2012. Modelling, predicting and mapping the emergence of aflatoxins in cereals in the EU due to climate change. Scientific report submitted to EFSA [Internet]. [place unknown]. Available from: http://www.efsa.europa.eu/en/supporting/pub/en-223

Bertuzzi T, Romani M, Rastelli S, Mulazzi A, Pietri A. 2017. Sterigmatocystin occurrence in paddy and processed rice produced in Italy in the years 2014–2015 and distribution in milled rice fractions. Toxins. 9(3):86.

Boddy L. 1984. The micro-environment of basidiomycete mycelia in temperate deciduous woodlands. In: Jennings DH, Rayner ADM, Eds. Ecology and physiology of the fungal mycelium. Cambridge: Cambridge University Press; p. 261–289.

Bokhari FM, Aly M. 2009. Evolution of traditional means of roasting and mycotoxins contaminated coffee beans in Saudi Arabia. Adv Biol Res (Rennes). 3(3–4):71–78.
Bünger J, Westphal G, Monnich A, Hinnendahl B, Hallier E, Müller M. 2004. Cytotoxicity of occupationally and environmentally relevant mycotoxins. Toxicology. 202:199–211.

Chemining’wa GN, Gathumbi JK, Njenga LN, Muthomi JW. 2009. The occurrence of aflatoxins in maize and distribution of mycotoxin-producing fungi in Eastern Kenya. Plant Pathol J. 8 (3):113–119.

Chen AJ, Frisvad JC, Sun BD, Varga J, Kocsube S, Dijksterhuis J, Kim DH, Hong SB, Houbraken J, Samson RA. 2016. Aspergillus section Nidulantes (formerly Entericella): polyphilic taxonomy, chemistry and biology. Stud Mycol. 84:1–118.

Crofton-Sleigh C, Doherty A, Ellard S, Parry EM, Venitt S. 1993. Micronucleus assays using cytchalasin-blocked MCL-5 cells, a proprietary human cell line expressing five human cytochromes P-450 and microsomal epoxide hydrolase. Mutagenesis. 8:363–372.

Culliao AGL, Barcelo JM. 2015. Fungal and mycotoxin contamination of coffee beans in Benguet province, Philippines. Food Additives & Contaminants: Part A. 32(2):250–260.

Curry PT, Reed RN, Martino RM, Kitchin RM. 1984. Induction of sister-chromatid exchanges in vivo in mice by the mycotoxins sterigmatocystin and griseofulvin. Mutat Res. 137:111–115.

Darwish WS, Bayomi RM, El-Moaty AM, Gad TM. 2009. Muscid contamination and aflatoxin residues in frozen chicken meat-cuts and giblets. Jpn J Vet Res. 64(2):167–171.

De Nijs M, Mengelers MJB, Boon PE, Heyndrickx E, Hoogenboom LAP, Lopez P, Mol HGI. 2016. Strategies for estimating human exposure to mycotoxins via food. World Mycotoxin Journal Special Issue: Mycotoxins in a changing world. 95(5):831–845.

EFSA Panel on Contaminants in the Food Chain (CONTAM). 2013. Scientific opinion on the risk for public and animal health related to the presence of sterigmatocystin in food and feed. EFSA J. 11(6):3254.

Egbuta M, Mulunda F, Njobeh PB, Phoku JZ, Chileka CA, Dutton MF, Dutton F. 2014. Isolation of filamentous fungi species contaminating some nigerian food commodities. J Food Res. 4(1):38–50.

El- Shanawany AA, Eman Mostafa M, Barakat A. 2005. Fungal populations and mycotoxins in silage in Assiut and Sohag governorates in Egypt, with a special reference to characteristic Aspergilli toxins. Mycopathologia. 159:281–289.

Essigmann JM, Barker LJ, Fowler KW, Francisco MA, Reinhold VN, Wogan GN. 1979. Sterigmatocystin-DNA interactions: identification of a major adduct formed after metabolic activation in vitro. Proc Natl Acad Sci U S A. 76:179–183.

Essigmann JM, Donahue PR, Story DL, Wogan GN, Brunengraber H. 1980. Use of the isolated perfused rat liver to study carcinogen-DNA adduct formation from aflatoxin B1 and sterigmatocystin. Cancer Res. 40:4085–4091.

European Food Safety Authority. 2010. Management of left-censored data in dietary exposure assessment of chemical substances. Eur Food Saf Auth J. 8:1–96.

Frisvad JC. 2015. Taxonomy, chemodiversity, and chemonconsistency of Aspergillus, Penicillium, and Talaromyces species. Front Microbiol. 5:773–783.

Gao W, Jiang L, Ge L, Chen M, Geng C, Yang G, Li Q, Ji F, Yan Q, Zou Y, et al. 2015. Sterigmatocystis-induced oxidative DNA damage in human liver-derived cell line through lysosomal damage. Toxicol Vitro. 29:1–7.

Greshock T, Grubbs AW, Jiao P, Wicklow DT, Gler JB, Williams RM. 2008. Isolation, structure elucidation, and biomimetic total synthesis of versicoloramide B, and the isolation of antipodal (+)-stephacidin A and (+)-nootoamide B from Aspergillus versicolor NRRL 35600. Angewandte Chemie. 47:3573–3577.

Haas D, Pfeifer B, Reiterich C, Partenheimer R, Reck B, Buzina W. 2013. Identification and quantification of fungi and mycotoxins from Pu-erh tea. Int J Food Microbiology. 166: (2):316–322.

Halstensen AS. 2008. Species-specific fungal DNA in airborne dust as surrogate for occupational mycotoxin exposure? Int J Mol Sci. 9:2543–2558.

Hammami W, Fiori S, Al Thani R, Ali Kali N, Balmas V, Mighei Q, Joua Q. 2014. Fungal and aflatoxin contamination of marketed spices. Food Control. 37:177–181.

Houbraken J, De Vries RP, Samson RA. 2014. Modern taxonomy of biotechnologically important Aspergillus and Penicillium species. Adv Appl Microbiol. 86:199–249.

Huang S, Wang J, Xing L, Shen H, Yan X, Wang J, Zhang X. 2014. Impairment of cell cycle progression by sterigmatocystin in human pulmonary cells in vitro. Food Chem Toxicol. 6689–95.

Huang X, Zhang X, Yan X, Yin G. 2002. Effects of sterigmatocystin on interleukin-2 secretion of human peripheral blood mononuclear cells in vitro. J Hyg Res. 31:112–114.

Hubka V, Nováková A, Kolarik A, Jurjevic Z, Peterson SW. 2014. Revision of Aspergillus section flavipes: seven new species and proposal of section Jani sect. Nov. Mycologia. 107(1):169–208.

Hubka V, Nováková A, Peterson SW, Stephen W, Frisvad JC, Sklenar F, Matsuzawa T, Kubátová A, Kolarik M. 2016. A reappraisal of Aspergillus section nidulantes with descriptions of two new sterigmatocystin-producing species. Plant Systematics and Evolution. 302:1267–1299.

In: IPCC, Pachauri RK, Reisinger A, ed. 2007. Climate change 2007: synthesis report. contribution of working groups i, ii and iii to the fourth assessment report of the intergovernmental panel on climate change. Geneva: IPCC.

Jukić Despot D, Kocsube S, Bencskí O, Kecskeméti A, Szekeres A, Vágvolgyi C, Varga J, Ševići Klič M. 2017. New sterigmatocystin-producing species of Aspergillus section Versicolum from indoor air in Croatia. Mycological Prog. 16(1):63–72.

Jiang X, Wang J, Xing L, Shen H, Lian Wu, Yi L, Zhang D, Yang H, Liu J, Zhang X. 2017. Sterigmatocystin-induced checkpoint adaptation depends on Chk1 in immortalized human gastric epithelial cells in vitro. Arch Toxicol. 91(9):259–270.

Jiao P, Mudur SV, Gler JB, Wicklow DT. 2007. Kupukasins, nucleoside derivatives from Aspergillus versicolor. J Nat Prod. 70:1308–1311.

Jurjevic Z, Peterson SW, Solfrizzo M, Peraica M. 2013. Sterigmatocystin production by nine newly described Aspergillus species in section Versicolum grown on two different media. Mycol Res. 29:141–145.

Kačanová M. 2003. Feeding soybean colonization by microscopic fungi. Trakya Univ J Sci. 4(2):165–168.

Kačanová M, Kňažovická V, Feštovičová S, Rovná K. 2012. Microscopic fungi recovered from honey and their toxigenicity. J Environ Sci Health. 47(11):1659–1664.
Kawai K, Nakamaru T, Nozawa Y, Maebayashi Y, Yamazaki M, Natori S. 1984. Inhibitory effect of sterigmatocystin and 5,6-dimethoxysterigmatocystin on ATP synthesis in mitochondria. Appl Environ Microbiol. 48:1001–1003.

Kelkar HS, Keller NP, Adams TH. 1996. Aspergillus nidulans stcP encodes an O-methyltransferase that is required for sterigmatocystin biosynthesis. Appl Environ Microbiol. 62:4296–4298.

Kocić-Tanackov SD, Đimić GR, Lević JT, Pejin DJ, Pejin JD, Jajić IM. 2010. Occurrence of potentially toxigenic mould species in fresh salads of different kinds of ready-for-use vegetables. APTEFF. 41:1–203.

Kovalenko AV, Soldatenko NA, Fetisov LN, Strel’tsov NV. 2011. More accurate determination of the minimum allowable level of sterigmatocystin in piglet feed. Russian Agric Sci. 37(6):504–507.

Labuda R, Tančinová D. 2006. Fungi recovered from Slovakian poultry feed mixtures and their toxigenicity. Ann Agric Environ Med. 13:193–200.

Lee YM, Li H, Hong J, Cho HJ, Bae KS, Kim MA, Kim D-K, Jung JH. 2010. Bioactive metabolites from the sponge-derived fungus Aspergillus versicolor. Arch Pharmacological Res. 33:231–235.

Lima CAP, Orsi R, Dilkin P, Correa B. 2000. Mycophora and aflatoxigenic species in derivatives of milled rice. Ciênc Tecnol Aliment. 20(1):37–39.

Liu Y, Du M, Zhang G. 2014. Proapoptotic activity of aflatoxin B1 and sterigmatocystin in HepG2 cells. Toxicol Rep. 1:1076–1086.

Magan N, Hope R, Cairns V, Aldred D. 2003. Post-harvest fungal ecology: impact of fungal growth and mycotoxin accumulation in stored grain. Eur J Plant Pathol. 109(7):723–730.

Magnoli C, Chiachiera SM, Mlazzo R, Palacio G, Angeletti A, Hallak C, Dalcero A. 2002. The mycoflora and toxicity of feedstuffs from a production plant in Cordoba, Argentina. Mycotoxin Res. 18(1):7–22.

Makun HA, Anjorin ST, Moronfuye B, Adejo FO, Afolabi OA, Fagbayibo G, Balogun BO, Surajudeen AA. 2010. Fungal and aflatoxin contamination of some human food commodities in Nigeria. Afr J Food Sci. 4(4):127–135.

McConnell IR, Garner RC. 1994. DNA adducts of aflatoxins, sterigmatocystin and other mycotoxins. In: IARC scientific publications. (125):49–55.

Miller JD, Sun M, Gilyan A, Roy J, Rand TG. 2010. Inflammation-associated gene transcription and expression in mouse lungs induced by low molecular weight compounds from fungi from the built environment. Chem Biol Interact. 183:113–124.

Miller JD, Trenholm L, eds. 1994. Mycotoxins in grain: compounds other than aflatoxin: ACCI Press.

Mo HGJ, Pietri A, MacDonald SJ, Anagnostopoulos C, Spanjer M. 2015. on sterigmatocystin in food. EFSA Supporting Publication. 12(3):774E.

Mol JGJ, De Rijk TC, Van Egmond H, De Jong J. 2014. Occurrence of mycotoxins and pesticides in straw and hay used as animal feed. Wageningen RIKILT Wageningen UR (RIKILT report 2014.006).

Mori H, Sugie S, Yoshimi N, Kitamura J, Niwa M, Hamasaki T, Kawai K. 1986. Genotoxic effects of a variety of sterigmatocystin-related compounds in the hepatocyte/DNA-repair test and the Salmonella microsome assay. Mutat Res. 173:217–222.

Nichea MJ, Palacios SA, Chiachiera SM, Sulyok M, Krška R, Chulze SN, Torres AM, Ramirez ML. 2015. Presence of multiple mycotoxins and other fungal metabolites in native grasses from a wetland ecosystem in Argentina intended for grazing cattle. Toxins. 7(8):3309–3329.

Pereira M, Radic B, Lucic A, Pavlovic M. 1999. Toxic effects of mycotoxins in humans. Who. 77:754–766.

Paterson RRM, Lima N. 2011. Further mycotoxin effects from climate change. Food Res Int [Internet]. 44:2555–2566. Available from: https://doi.org/10.1016/j.foodres.2011.05.038

Piecková E, Hurbánková M, Cermá S, Majorosová M, Kováčiková Z, Pangallo D. 2011. Respiratory toxicity of Aspergillus versicolor: the most common indoor mould in Slovakia. In: Brebbia CA, Egliie M, Knets I, Of R M, Popov V, Eds. Environmental health and biomedicine 15. Southampton: Wit Press; p. 135–145.

Piecková E. 2015. Domestic environment – indoor mycobacteria as a public health risk factor. In: Viegas C, Pinheiro C, Sabino R, Viegas S, Brandao J, Verissimo C, Eds. Environmental mycology in public health. Fungi and mycotoxins risk assessment and management. Elsevier – Academic Press; p. 129–146. https://www.elsevier.com/books/environmental-mycology-in-public-health/viegas/978-0-12-411471-5

Piotrowska M. 2013. Contamination of breakfast cereal products by fungi and mycotoxins – a potential risk for consumer’s health. Biotechnol Food Sci. 77(1):3–10.

Purchase IF, Van Der Watt JJ. 1969. Acute toxicity of sterigmatocystin to rats. Food Cosmet Toxicol. 7:135–139.

Rank C, Nielsen LF, Larsen TO, Varga J, Samson RA, Frisvad JC. 2011. Distribution of sterigmatocystin in filamentous fungi. Fungal Biol. 115:406–420.

Raper KB, Fennel DI. 1965. The genus Aspergillus: Williams and Wilkins Company, Baltimore. 686.

Reddy MV, Irvin TR, Randerath K. 1985. Formation and persistence of sterigmatocystin DNA adducts in rat-liver determined via P-32-postlabeling analysis. Mutat Res. 152:85–96.

Riba A, Mokrane S, Mathieu F, Lebríhi A, Sabau N. 2008. Mycophora and ochratoxin A producing strains of Aspergillus in Algerian wheat. Int J Food Microbiol. 122(1–2):85–92.

Rofiat AS, Fanelli F, Atanda O, Sulyok M, Cozzi G, Bavaro S, Krška R, Logriece AF, Ezekiel CN. 2015. Fungal and bacterial metabolites associated with natural contamination of locally processed rice (Oryza sativa L.) in Nigeria. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 32(6):950–959.

Rosa CAR, Cavaglieri LR, Ribeiro JMM, Keller KM, Alonso VA, Chiachiera SM, Dalcero AM, Lopes CWG. 2008. Mycobiota and naturally-occurring ochratoxin A in dairy cattle feed from Rio de Janeiro State, Brazil. World Mycotoxin Journ. 1(2):195–203.

Sabino R, Faisca VM, Carolino E, Veríssimo C, Viegas C. 2012. Occupational exposure to aspergillus by swine and poultry farm workers in Portugal. J Toxicol Environ Health. 75:1381–1391.

Samson RA, Houbraken J, Thrane U, Frisvad JC, Andersen B. 2010. Food and indoor fungi: CBS-KNAW fungal biodiversity centre. Utrecht. 390.

Samson RA, Visagie CM, Houbraken J, et al. 2014. Phylogenetic, identification and nomenclature of the genus aspergillus. Studies in Mycology. 78:141–173.
Sawane A, Sawane M. 2014. Mycotoxicogenicity of Aspergillus. *Penicillium fusarium* spp. isolated stored rice. Int J Curr Microbiol Appl Sci. 3(11):116–121.

Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CV, Chen W, Consortium FB. 2012. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for fungi. In: Janzen DH, Ed. Proceedings of the national academy of sciences of the United States of America, Philadelphia; p. 6241–6246.

Silva JB, Pozzi CR, Mallozi MAB, Ortega EM, Corre B. 2000. Mycoflora and occurrence of aflatoxin B1 and fumonisin B1 during storage of Brazilian sorghum. J Agric Food Chem. 48:4352–4356.

Sivakumar V, Thanislass J, Niranjali S, Devaraj H. 2008. Aspergillus, Penicillium, Talaromyces and occurrence of aflatoxins in cereals in South-Western Romania. Res J Agric Sci. 42(2):42–47.

Sonjak S, Ličena M, Frisvad JC, Gunde-Cimerman N. 2011. The mycobiota of three dry-cured meat products from Slovenia. Food Microbiol. 28(3):373–376.

Tabuc C, Marin D, Guerre P, Susa T, Bailly JD. 2009. Molds and mycotoxin content of cereals in Southeastern Romania. J Food Prot. 72(3):662–665.

Tabuc C, Stroia C, Neacsu A. 2010. Incidence of *Aspergillus* strains and of aflatoxin B1 in cereals in South-Western Romania. Res J Agric Sci. 42(2):42–47.

Tabuc C, Taranu I, Calin L. 2011. Survey of moulds and mycotoxin contamination of cereals in South-Eastern Romania in 2008–2010. Archiva Zootecnica. 14(4):25–38.

Tančínová D, Barboráková Z, Kačinová J, Mašková Z, Volčková M. 2013. The occurrence of micromycetes in apples and their potential ability to produce mycotoxins. J Microbiology, Biotechnol Food Sci. 2(Special issue 1):1800–1803.

Tančínová D, Labuda R. 2009. Fungi on wheat bran and their toxigenicity. Ann Agric Environ Med. 16:325–331.

Toffa DD, Mahnine N, Ouaffak L, Al Abidi A, El Alaoui Faris FZ, Zinedine A. 2013. First survey on the presence of ochratoxin A and fungi in raw cereals and peanut available in the Republic of Niger. Food Control. 32:558–562.

Townsend CA. 1997. Progress towards a biosynthetic rationale of the aflatoxin pathway. Pure Appl Chem. 58:227–238.

Ueda N, Fujie K, Gotoh-Mimura K, Chattopadhyay SC, Sugiyama T. 1984. Acute cytogenetic effect of sterigmatocystin on rat bone-marrow cells in vivo. Mutat Res. 139:203–206.

Ueno Y, Umemori K, Niimi E, Tanuma S, Nagata S, Sugamata M, Ihara T, Sekijima M, Kawai K, Ueno I, et al. 1995. Induction of apoptosis by T2 toxin and other natural toxins in HL-60 human promyelocytic leukemia cells. Nat Toxins. 3(1):129–137.

Van der Fels-Klerx HJ, Liu C, Battilani P. 2016. Modelling climate change impacts on mycotoxic contamination. World Mycotoxin J [Internet]. 9:1–10. Available from: http://www.wageningenacademic.com/doi/10.3920/WMJ2016.2066

Veršilovskis A, Bartkevičiūtė V. 2012. Stability of sterigmatocystin during the bread making process and its occurrence in bread from the Latvian market. Mycotoxin Res. 28:123.

Veršilovskis A, Bartkevičiūtė V, Mikėlsone V. 2008a. Sterigmatocystin presence in typical Latvian grains. Food Chemistry 109 (1):243–248.

Veršilovskis A, De Saeger S, Mikelsone V. 2008b. Determination of sterigmatocystin in beer by high performance liquid chromatography with ultraviolet detection. World Mycotoxin Journal. 1(2):161–166.

Veršilovskis A, Van Peteghem C, De Saeger S. 2009. Determination of sterigmatocystin in cheese by high-performance liquid chromatography–tandem mass spectrometry. Food Additives & Contaminants: Part A. 26(1):127–133.

Viegas C, Pacifico CFaria T. 2017. Fungal contamination in green coffee beans samples: a public health concern. Journal Of Toxicology and Environmental Health, Part a. doi:10.1080/15287394.2017.1286927

Viegas C, Sabino R, Viegas S, Veríssimo C. 2013. Occupational exposure to toxicogenic *Aspergillus versicolor* in Portuguese swine. Int Symp Occup Saf Hyg. 3: 433–434.

Viegas S, Veiga L, Figueiredo P, Almeida A, Carolino E, Viegas C. 2015. Assessment of workers’ exposure to aflatoxin B1 in a Portuguese waste industry. Ann Occup Hyg. 59(2):173–181.

Visagie CM, Hirooka Y, Tanney JB, Whitfield E, Mwange K, Meijer M, Amend AS, Seifert KA, Samson RA. 2014. *Aspergillus, Penicillium* and *Talaromyces* isolated from house dust samples collected around the world. Stud Mycol. 78(6):139–146.

Walkow J, Sullivan G, Maness D, Yakatan GJ. 1985. Sex and age differences in the distribution of 14-C-sterigmatocystin in immature and mature rats: a multiple dose study. J Am Coll Toxicol. 4:45–51.

Wang J, Huang S, Xing L, Cui J, Tian Z, Shen H, Jiang X, Yan X, Wang J, Zhang X. 2015. Sterigmatocystin induces G1 arrest in primary human esophageal epithelial cells but induces G2 arrest in immortalized cells: key mechanistic differences in these two models. Arch Toxicol. 89(11):2015–2025.

Warth B, Parich A, Atehnikeng J, Bandypadhyay R, Schuhmacher R, Sulyok M, R.K. 2012. Quantitation of mycotoxins in food and feed from burkinafaso and Mozambique using a modern LC-MS/MS multitoxin method. J Agric. Food Chem. 60(36):9352–9363.

Xie T-X, Misumi J, Aoki K, Zhao W-Y, Liu S-Y. 2000. Absence of p53-mediated G1 arrest with induction of MDM2 in sterigmatocystin-treated cells. Int J Oncol. 17:737–742.

Xing LX, Zhang XH, Li YH, Yan X, Wang J, Wang F. 2005. Effects of sterigmatocystin on HLA-1 expression of human peripheral blood mononuclear cells in vitro. J Hyg Res. 34:454–456.

Yassin MA, Ama E-S, Moslem MA, El-Naggar MA. 2013. Mycobiota of almond seeds and the toxigenicity of some involved genera. Life Sci J. 10(4):1088–1093.

Youssef MS, Omo E-M, Ibrahim YM. 2008. Mycobiota and mycotoxins of Egyptian peanut (*Arachis hypogaea*) seeds. Int J Bot. 4(4):349–360.

Yu J, Chang P-K, Ehrlich KC, Cary JW, Bhatnagar D, Cleveland TE, Payne GA, Linz JE, Woloshuk CP, Bennett JW. 2004. Clustered pathway genes in aflatoxin biosynthesis. Appl Environ Microbiol. 70:1253–1262.

Zhang Y, Yao ZG, Wang J, Xing LX, Xia Y, Zhang XH. 2012. Effects of sterigmatocystin on TNF-alpha, IL-6 and IL-12 expression in murine peripheral blood mononuclear cells and peritoneal macrophages in vivo. Mol Med Rep. 5:1318–1322.