European Food Safety Authority

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SCIENTIFIC OPINION

Scientific Opinion on the risk for public and animal health related to the presence of sterigmatocystin in food and feed[1]

EFSA Panel on Contaminants in the Food Chain (CONTAM)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The European Food Safety Authority (EFSA) was asked by the European Commission to deliver a scientific opinion on sterigmatocystin (STC) in food and feed. STC is a polyketide mycotoxin that shares its biosynthetic pathway with aflatoxins. Following an EFSA call for data, analytical results from 247 food and 334 feed samples were submitted. In food, analytical results on STC were reported to be all below the limit of detection or limit of quantification. In feed, only four quantified results were reported. Therefore, the EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) concluded that the available occurrence data are too limited to carry out a reliable human and animal dietary exposure assessment. Acute oral toxicity of STC is relatively low, and liver and kidneys are the target organs. STC is mutagenic in both bacterial and mammalian cells after metabolic activation and forms DNA adducts. Tumourigenicity has been observed after oral, intraperitoneal, subcutaneous and dermal administration resulting in hepatocellular carcinomas, haemangiosarcomas in the liver, angiosarcomas in brown fat and lung adenomas. Since no exposure data were available, the margin of exposure approach for substances that are genotoxic and carcinogenic could not be applied for STC, and thus the CONTAM Panel could not characterise the risk for human health. Regarding animals, the Panel noted that STC is hepatotoxic in poultry and pigs, and nephrotoxic in poultry and toxic in several fish species. However, in the absence of exposure data for livestock, fish and companion animals, and given the limited knowledge on the adverse effects of STC, the CONTAM Panel could not characterise the risk for animal health. More occurrence data on STC in food and feed need to be collected to allow dietary exposure assessment. For food, methods with a limit of quantification of less than 1.5 µg/kg should be applied.

KEY WORDS

mycotoxins, sterigmatocystin, food, feed, dietary exposure, genotoxic and carcinogenic, risk assessment.

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SUMMARY

The European Commission asked the European Food Safety Authority (EFSA) to provide a scientific opinion on the risks for public and animal health related to the presence of sterigmatocystin (STC) in food and feed.

STC is a polyketide mycotoxin that is produced by more than 50 fungal species, including *Aspergillus flavus*, *A. parasiticus*, *A. versicolor* and *A. nidulans*, of which *A. versicolor* is the most common source. STC shares its biosynthetic pathway with aflatoxins. *A. nidulans* and *A. versicolor* are apparently unable to biotransform STC into O-methylsterigmatocystin, the direct precursor of aflatoxin B_1 (AFB_1) and G_1 (AFG_1). Consequently, substrates colonised by these fungi can contain high amounts of STC, while substrates invaded by *A. flavus* and *A. parasiticus* contain only low amounts of STC as most is converted into AFs. STC can occur in grains and grain-based products due to fungal infestation at the post-harvest stage.

Analytical methods for STC in food and feed based on liquid chromatography-mass spectrometry (LC–MS) reach the lowest limits of detection (LOD). However, the sensitivity varies between individual matrices and whether a single or multi-analyte method is applied. So far, no certified reference materials are available for STC. In addition, no proficiency tests for STC analysis in food or feed could be identified.

In the literature, only limited information is available on the occurrence of STC in different foods and feed. Most of the earlier studies using methods with rather high LODs/limits of quantification (LOQ) report a high percentage of left-censored data (often 100 %) on food and feed. Two recent studies using a liquid chromatography-tandem mass spectrometry (LC–MS/MS) method with an LOD of 0.15 µg/kg and an LOQ of 0.3 µg/kg reported STC positive samples of bread and of grain intended for use as animal feed. While these data indicate that these methods are sufficiently sensitive to quantify STC in food and feed, the data were insufficient to use for a reliable exposure assessment.

Moreover, STC has been occasionally reported to occur in green coffee beans, spices, nuts and beer. Contamination of cheese occurs particularly at the surface, following fungal spoilage during ripening and storage. Food processing (e.g. milling, bread and cheese making, roasting) can result in a decrease of the STC concentration, however the extent depends on the type of food and the processing conditions.

Following an EFSA call for data, analytical results from 247 food samples submitted by two Member States were reported to be all below the LOD or LOQ. The EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) concluded that a reliable exposure assessment was not feasible for humans.

The scarce information available suggests that absorption of STC is limited following oral exposure. STC is metabolised in the liver and lung by various cytochrome P450 enzymes into different hydroxymetabolites and its reactive exo-epoxide that readily forms DNA adducts. Excretion of both conjugated parent STC and its hydroxylated metabolites occurs via bile and urine.

There are insufficient data to assess the rate of carryover of STC into milk when ruminants are exposed to contaminated feed. Moreover, no information is available about the transfer of STC and/or its metabolites into other animal products such as meat and eggs.

The acute oral toxicity of STC is relatively low with LD_{50} values varying between 120 and 166 mg/kg body weight (b.w.). Liver and kidneys are the target organs of acute toxicity. Liver lesions are observed in rat, mouse, monkey and guinea pig and increase with dose and duration of exposure. They are defined as hepatocellular necrosis and haemorrhages. In the kidney, hyaline degeneration, tubular necrosis and haemorrhages were described in rats and/or vervet monkeys (*Chlorocebus pyreguthreus*) exposed to STC. Results from *in vivo* and *in vitro* studies suggest that STC may have immunomodulatory activity, but firm conclusions cannot be drawn.

STC is mutagenic in both bacterial and mammalian cell assays after metabolic activation. It induces chromosomal damage both *in vitro*, upon metabolic activation, and *in vivo* in experimental animals. 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. It is concluded that STC is genotoxic and carcinogenic.

Despite the evidence on genotoxicity and carcinogenicity, only a limited tumourigenicity database was available for dose–response assessment. From the data available on haemangiosarcomas in the liver of male rats, a benchmark dose for a response of 10 % extra risk (BMD_{10}) of 0.36 and a lower 95 % confidence limit for a benchmark response of 10 % extra risk ($BMDL_{10}$) of 0.16 mg STC/kg b.w. per day were calculated. A comparison of the BMD_{10} of STC for the occurrence of haemangiosarcomas and that of AFB_1 for the occurrence of HCC suggested that the carcinogenic potency of STC is approximately three orders of magnitude lower than that of AFB_1.

Due to the absence of exposure data for the European population, the margin of exposure (MOE) approach for substances that are genotoxic and carcinogenic could not be applied for STC and therefore, the CONTAM Panel could not characterise the risk of STC for human health.

Concerning feed contamination, following an EFSA call for data, analytical results from 334 feed samples were submitted by two Member States. Except for four samples, analytical results on STC were reported to be all below the LOD or LOQ. Based on the available data, the CONTAM Panel concluded that a reliable exposure assessment was not feasible for animals.

The toxicity of STC in livestock, fish and companion animals remains largely unknown. In sheep, no signs of toxicity were observed in a feeding trial at the highest dose tested (16 mg STC/kg feed, estimated as equivalent to 0.3 mg/kg b.w. per day). Only limited data are available for other ruminants, but a case report describes haemorrhages and bloody diarrhoea in cattle following exposure to STC. STC is hepatotoxic in poultry and pigs, and nephrotoxic in poultry. Toxicity of STC has been demonstrated in several fish species. Pathological alterations included haemorrhages and oedema of the gills and haemorrhages and eosinophilic infiltrations in the hepatopancreas. No data on adverse effects of STC could be identified for other animal species. In the absence of exposure data for livestock, fish and companion animals, and given the limited knowledge on the adverse effects of STC following the ingestion of contaminated feed, the CONTAM Panel could not characterise the risk of STC for animal health.

The EFSA Scientific Committee has concluded that an MOE of 10 000 or more for substances that are genotoxic and carcinogenic is of low concern for public health. Based on the $BMDL_{10}$ (0.16 mg STC/kg b.w. per day) and an MOE of 10 000, the STC concentration in grains and grain-based products leading to an exposure of low health concern (0.016 µg/kg b.w. per day) would range from 1.5 to 8 µg/kg. The available information on toxicity and exposure in farm and companion animals is insufficient to draw similar conclusions for feed.

Since currently, due to the absence of exposure data, a risk characterisation is not possible for STC, the CONTAM Panel recommends that more occurrence data for STC in food and feed across European countries need to be collected. For food, methods with an LOQ of less than 1.5 µg/kg should be applied, whereas for feed, the available information is insufficient to make a recommendation. The development of suitable certified reference materials and/or proficiency tests to support analytical methodology should be encouraged.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

Sterigmatocystin is mainly produced by the fungi *Aspergillus nidulans* and *Aspergillus versicolor*. It has been reported in cereals, green coffee beans and cheese. Sterigmatocystin is a mycotoxin closely related to aflatoxins.

AVAILABLE INFORMATION (NOT EXHAUSTIVE)

In accordance with Article 36 of Regulation (EC) No 178/2002, a report "Scientific information on mycotoxins and natural plant toxicants" has been produced following a grant agreement between the European Food Safety Authority (EFSA) and the author(s) of the report (CFP/EFSA/CONTAM/2008/01). The report presents information, inter alia, regarding sterigmatocystin in feed and food and is available on the EFSA website (http://www.efsa.europa.eu/en/scdocs/doc/24e.pdf)

ISSUE

There might be a possible risk for animal and public health, related to the presence of sterigmatocystin in feed and food. The European Commission asks EFSA to assess on the basis the available information the risk for animal and public health in order to enable the European Commission and the competent authorities in the Member States to consider the need for a possible follow up including to fill the knowledge gaps.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

In accordance with Art. 29 (1) of Regulation (EC) No 178/2002, the European Commission asks the European Food Safety Authority to provide a scientific opinion on the risks for animal and public health related to the presence of sterigmatocystin in feed and food.

The assessment should, based upon the available information, assess if the presence of sterigmatocystin in food and feed is a potential risk for public and animal health taking into account the toxicity of sterigmatocystin and the occurrence in feed and food. For the assessment of the risks related to the presence of sterigmatocystin in feed, the situation for the different farm animal species should be considered. For the assessment of the risks related to the presence of sterigmatocystin in food the situation for the specific (vulnerable) groups of the human population (e.g. high consumers, children, people following specific diets, etc.) should be considered.

ASSESSMENT

1. Introduction

Sterigmatocystin (STC) is a polyketide mycotoxin that is produced by several fungal species, including *Aspergillus flavus*, *A. parasiticus*, *A. versicolor* and *A. nidulans*, of which *A. versicolor* is the most common source. A recent assessment of fungi for potential STC biosynthesis has provided a comprehensive list of 55 fungal species within the genera *Emericella*, *Aspergillus*, *Chaetomium*, *Botryotrichum* and *Humicola* that share the ability to produce STC (Rank et al., 2011). Previous reports that some *Penicillium* spp. produce STC have not been confirmed by this recent study. STC shares its biosynthetic pathway with aflatoxins (AF) (Sweeney and Dobson, 1999; see also Appendix A). Wild-type *A. nidulans* and *A. versicolor* are apparently unable to biotransform STC into O-methylsterigmatocystin, the direct precursor of aflatoxin B_1 (AFB_1) and G_1 (AFG_1) (Sweeney and Dobson, 1999; Yu et al., 2004). Consequently, substrates colonised by these fungi can contain high amounts of STC, while substrates invaded by *A. flavus* and *A. parasiticus* contain only low amounts of STC as most is converted into AFs (Yu et al., 2004).

A. vesicolor is able to grow on substrates with a low water activity (a_w between 0.75 and 0.95) and in a temperature range between 4 and 40 °C, with an optimum for toxin production at temperatures between 23 and 29 °C (Betina, 1989).

Grains and grain-based products have been found to contain STC due to fungal infestation at the post-harvest stage (storage, transport and processing). STC has been measured incidentally in other products of plant origin such as green coffee beans, spices and beer. In addition, fungal spoilage and production of STC has been observed in cheese.

Owing to the structural similarities, AFs and STC share prominent toxic effects, including genotoxicity and carcinogenicity (Miller and Trenholm, 1994; see Section 7.2.5). However, in contrast to AFs, only limited information on occurrence and toxicity of STC is available.

1.1. Previous assessments

The International Agency for Research on Cancer (IARC) has assessed the carcinogenic potential of STC (IARC, 1976, 1987) and concluded that STC produced lung tumours in mice and liver tumours in rats following oral administration. The IARC noted that in rats, STC induced skin and liver tumours following its administration to the skin and sarcomas at the site of its subcutaneous (s.c.) injection. No case reports or epidemiological studies were available for evaluation by IARC and it was concluded that STC is possibly carcinogenic to humans (group 2B).

The California Environmental Protection Agency (1992, 2000) has evaluated the human cancer potency of STC based on five datasets comprising liver tumours in male rats (Ohtsubo et al. 1978; Maekawa et al., 1979), hepatocellular carcinomas (HCC) in rats (sexes combined) treated by gavage and feeding (Purchase and van der Watt, 1970a), and liver carcinomas in male rats (Terao et al., 1978). A no significant risk level of 0.02 µg per day was derived as the intake associated with a lifetime cancer risk of 10^{-5} or lower for a 70 kg body weight (b.w.) adult.

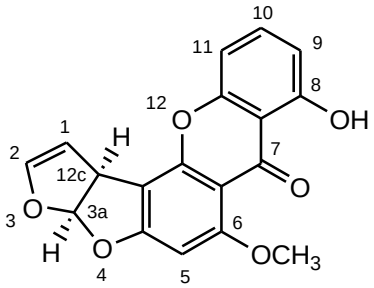
The approach taken by EFSA to characterise the risk of substances that are genotoxic and carcinogenic is the margin of exposure (MOE) approach, in which no implicit assumptions about a 'safe' intake (e.g. a no significant risk level) or a lifetime cancer risk are made (EFSA, 2005).

1.2. Chemistry

STC ((3aR,12cS)-3a,12c-dihydro-8-hydroxy-6-methoxy-7H-furo[3',2':4,5]furo[2,3-c]xanthen-7-one; $C_{18}H_{12}O_6$, molecular mass 324.28 g/mol; CAS No 10048-13-2) is a polyketide mycotoxin structurally related to AFB_1 (Figure 1). STC crystallises as pale-yellow needles with a melting point of

245-246 °C. It is methylated at the C8-hydroxyl group by methyl sulphate or methyl iodide. Methanol or ethanol in acid, form dihydroalkoxysterigmatocystin from STC (Lou et al., 1994; Versilovskis and De Saeger, 2010). Ultraviolet (UV) spectrum (in ethanol): 235 nm (extinction coefficient (ε) = 24 500), 249 nm (ε = 27 500), and 329 nm (ε = 13 100) (Cole and Schweikert, 2003). STC is characterised by a weak fluorescence (Maness et al., 1976).

Sterigmatocystin



Aflatoxin B_1

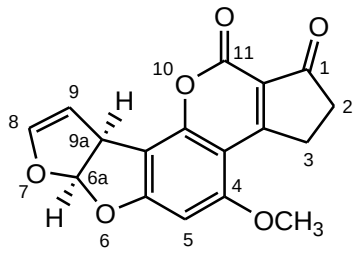


Figure 1: Chemical structures of sterigmatocystin and aflatoxin B_1.

STC is readily soluble in chloroform, as well as other organic solvents, such as methanol, ethanol and acetonitrile (Septien et al., 1993; Versilovskis and De Saeger, 2010). Septien et al. (1994) also showed the low solubility of STC in aqueous solutions (phosphate buffer) at different pH values.

Septien et al. (1993) observed a stability of more than 95 % in chloroform at 4 °C and at -20 °C for 30 days. No other data on stability of STC could be identified.

2. Legislation

In order to protect public health, Article 2 of Council Regulation (EEC) No 315/93[4] stipulates that, where necessary, maximum tolerances for specific contaminants shall be established. Therefore, a number of maximum levels (MLs) for contaminants as well as natural plant toxicants in food commodities are currently laid down in Commission Regulation (EC) No 1881/2006[5]. To ensure agricultural productivity and sustainability, animal and public health, animal welfare and protection of the environment, maximum contents for undesirable substances in animal feed are laid down in the EU Directive 2002/32/EC of the European Parliament and the Council of 7 May 2002[6]. While MLs for various mycotoxins were set for a number of food commodities and for AFs in feedingstuffs, the occurrence of STC is not regulated so far under these or other Regulations within the European Union (EU).

MLs for STC have been previously reported from the Czech Republic and Slovakia by the Food and Agriculture Organization (FAO) (FAO, 2004). However, both countries withdrew their legislation on STC when becoming a member of the European Union.

3. Methods of analysis

Analytical methods for STC have recently been reviewed by Versilovskis and De Saeger (2010). This includes a comprehensive overview of chromatographic methods for the detection of STC in foodstuffs, feed, dust, building and indoor air. STC reference standards can be obtained from several commercial suppliers. ^{13}C labelled STC is commercially available which could be used as an internal standard for mass-spectrometry based methods to compensate for analyte losses during extraction,

[4] Council Regulation (EEC) No 315/93 of 8 February 1993 laying down Community procedures for contaminants in food. OJ L 37, 13.2.1993, p. 1–3.
[5] Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. OJ L 364, 20.12.2006, p. 5–24.
[6] Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed. OJ L 140, 30.5.2002, p. 10–21.

stability issues during analysis and matrix effects. No certified reference materials are available. However, Tanaka et al. (2008) reported the preparation of a homogeneous brown rice reference material containing STC. No proficiency tests for STC analysis in food or feed could be identified and so far only one thin-layer chromatographic (TLC) method has been officially validated[7].

3.1. Sample collection and storage

Fungal growth and mycotoxin production are significantly affected by crop variety, agronomic practices, weather conditions during growth and harvest, storage and processing conditions as well as toxigenic potential of individual mould species. Consequently, the distribution of mycotoxins in a lot of agricultural products can be very heterogeneous if fungal invasion occurs post harvest during storage ('hot spots'). Therefore, sampling is the largest source of variability associated with mycotoxin test procedures and the most crucial step in obtaining reliable results (Köppen et al., 2010). The European Commission established sampling methods to be used for the official control of mycotoxins in cereals and cereal-based products, dried fruit, dried figs, (ground)nuts, spices, milk and milk products (including milk products for infants), coffee and coffee products, fruit juices, cider and wine, apple products and apple juice, and baby food (Regulation 401/2006/EC[8]). No specific research on sampling plans has been undertaken for STC, but the above-mentioned procedures provide a valuable guidance for sampling also for STC analyses.

3.2. Methods of analysis of sterigmatocystin

3.2.1. Sample preparation

Methods for STC extraction from food and feed are mainly based on a mixture of acetonitrile (or methanol) and aqueous potassium chloride, but also chloroform and ethyl acetate were described as extraction solvents. For defatting of samples, *n*-hexane is applied while sample clean-up is performed by liquid–liquid extraction (mostly with chloroform). In addition, solid-phase extraction (SPE) on different SPE columns has been described (Versilovskis and De Saeger, 2010). Immunoaffinity columns are not available for STC clean-up.

Liquid chromatography-tandem mass spectrometry (LC–MS/MS) multi-mycotoxin methods that include STC are frequently based on a single extraction step for all investigated mycotoxins (e.g., acetonitrile/water mixture) followed by the direct analysis of the diluted crude extract (Sulyok et al., 2007; Spanjer et al., 2008). This so-called dilute-and-shoot approach requires very sensitive analytical instruments and often suffers from considerable matrix effects.

In the case of analysis of samples of the indoor environment, many different extraction methods are described, depending on the sample type (air, dust, mycelium, building materials). Several extraction solvents, e.g., acetonitrile, methanol/water, dichloromethane/ethyl acetate, ethyl acetate, are used, followed by defatting, filtration, centrifugation and/or evaporation steps (Tuomi et al., 2000; Engelhart et al., 2002; Delmulle et al., 2006; Bloom et al., 2007; Hintikka et al., 2009).

3.2.2. Instrumental techniques

STC detection in food and feed extracts is performed by immunochemical or preferably by chromatographic techniques. Table 1 summarises the characteristics and the limits of detection (LOD) of the different analytical methods.

3.2.2.1. Immunochemical methods

Only a very limited number of enzyme-linked immunoassay (ELISA) methods have been described for STC, making use of either polyclonal (Morgan et al., 1986; Olson and Chu, 1993a) or monoclonal

[7] AOAC Official Method 973.38 for sterigmatocystin in barley and wheat: AOAC Official Methods of Analysis, on-line edition, chapter 49.

[8] Commission Regulation (EC) No 401/2006 of 23 February 2006 laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs. OJ L 70, 9.3.2006, p. 12-34.

antibodies (Li et al., 1996). In wheat, the lowest reproducible concentration of STC that could be quantified was 31 µg/kg, with a working range up to 250 µg/kg (Li et al., 1996).

3.2.2.2. Chromatographic techniques

a. Thin-layer chromatography (TLC) with fluorescence detection (FLD) is commonly reported for STC analysis of extracts from different food and feed matrices with LODs ranging from 2 to 140 µg/kg (Versilovskis and De Saeger, 2010). A validated official method is based on TLC with $AlCl_3$ spraying. The limit of quantification (LOQ) was 30 µg/kg in maize and 100 µg/kg in wheat and barley[7] (Stack and Rodricks, 1971, 1973).

b. Gas chromatography (GC) methods for STC determination are scarce with reported LODs at 5 µg/kg in grains (Salhab et al., 1976) and 50 µg/kg in rice (Manabe et al., 1973).

c. High-performance liquid chromatography (HPLC) methods for STC determination are described as single analyte methods (Stack et al., 1976; Schmidt et al., 1981; Abramson and Thorsteinson, 1989; Scudamore et al., 1998a; Tanaka et al., 2007; Versilovskis et al., 2008b) as well as in multi-mycotoxin methods (Hurst et al., 1987; Tangni and Pussemier, 2007). Detection is performed by UV (including diode array detection, DAD) or FLD. HPLC methods are applied to extracts of different food, feed and grain dust matrices with LODs ranging from 0.3 to 100 µg/kg (Scudamore et al., 1998a; Tangni and Pussemier, 2007; Versilovskis et al., 2008b).

d. Liquid chromatography-tandem mass spectrometry (LC–MS/MS) methods with low limits of detection are described for detection of STC in different food and feed matrices. Scudamore et al. (1996) described the determination of STC at levels down to less than 5 µg/kg in cheese, bread and maize products by HPLC with atmospheric pressure ionisation mass spectrometric detection. For grains, a sensitive LC-electrospray positive ionisation (ESI^+)–MS/MS method was developed and validated for STC determination with the lowest validation level at 0.5 µg/kg (Versilovskis et al., 2007). This LC–MS/MS method was modified and applied to the analysis of STC in cheese samples by Versilovskis et al. (2009) and reached the low detection limit of 0.03 µg/kg.

STC has been included in several multi-mycotoxin liquid chromatography-mass spectrometry (LC–MS) methods applied to extracts from mouldy food samples, pistachios, sweet peppers, food supplements, beer and cereal grains with LODs ranging from 0.4 to 10 µg/kg (Sulyok et al., 2007; Spanjer et al., 2008; Di Mavungu et al., 2009; Monbaliu et al, 2009; Martos et al., 2010; Zachariasova et al., 2010).

Also for indoor environments and building materials, STC detection is mainly performed by use of single or multi-mycotoxin LC–MS/MS methods (Tuomi et al., 2000; Engelhart et al., 2002; Delmulle et al., 2006; Bloom et al., 2007; Hintikka et al., 2009; Vishwanath et al., 2011) with LODs ranging between 1 ng/mL fungal culture sample (Delmulle et al., 2006) and 6 ng/g dust sample (Hintikka et al., 2009).

Table 1: Overview of characteristics and limits of detection of most important sterigmatocystin analytical methods in food and feed.

Analytical technique	Method characteristics	LOD (µg/kg)
ELISA	Screening (qualitative - semi-quantitative)	n.r.[(a)]
TLC	Screening (qualitative – semi-quantitative)	2–140
GC	Confirmation (semi-quantitative – quantitative)	5–50
HPLC with UV detection (including DAD) or FLD	Confirmation (semi-quantitative – quantitative)	
LC-MS[(b)]	Possible multi-analyte detection	0.3–100

ELISA: enzyme-linked immunosorbent assay; TLC: thin-layer chromatography; GC: gas chromatography; HPLC: high-performance liquid chromatography; UV: ultraviolet; DAD: diode array detection; FLD: fluorescence detection; LC–MS: liquid chromatography–mass spectrometry; LOD: limit of detection; LOQ: limit of quantification; n.r.: not reported.

(a): An LOQ of 31 µg/kg was reported by Li et al. (1996).

(b): LC–MS can be used for indoor environments analysis as well, with LODs reported in the range of 1 to 6 ng/mL or ng/g sample.

3.3. Concluding remarks

Analytical methods for STC in food and feed based on LC –MS reach the lowest LODs. However, the sensitivity varies between individual matrices and whether a single or multi-analyte method is applied. So far, no certified reference materials are available for STC. In addition, no proficiency tests for STC analysis of food or feed could be identified.

4. Occurrence of sterigmatocystin in food and feed

4.1. Previously reported literature data on the occurrence of sterigmatocystin

During the last 30–40 years only a limited number of surveys on the occurrence of STC in different foods were carried out. A recent review by Versilovskis and De Saeger (2010) described occurrence of STC in foodstuffs in general and in cheese in particular.

The toxin has been reported in grains, nuts, green coffee beans, spices, beer and cheese. It should be noted that not all studies reported LOD and LOQ values, meaning that negative findings must be interpreted with caution. Appendix B gives further details on the studies, including the LODs and LOQs as far as reported. Whether unprocessed cereal grains are intended for food or feed use, remains often unclear and they are therefore discussed in a separate section.

4.1.1. Occurrence of sterigmatocystin in grains

Appendix B (Table B1) gives an overview of published occurrence data in grains. The first surveys on the occurrence of STC have been carried out applying TLC as an analytical technique. In Europe such surveys were reported for Germany (Thurm et al., 1979), Poland (Szebiotko et al., 1981), the United Kingdom (UK) (Buckle, 1983), Czechoslovakia (Bartos and Matyas, 1983), Bulgaria (Petkova-Bocharova et al., 1991) and Turkey (Ozay and Heperkan, 1989). Grains included maize, barley, wheat, oats and rye. STC was not found in most of the tested grain samples. However LODs and LOQs were high (≥ 15 µg/kg) or were not reported.

In recent years, there has been significant progress in developing more precise analytical techniques. In particular, HPLC methods were applied for the determination of STC in various commodities. In the UK, four surveys have been carried out on the occurrence of STC in grains using HPLC. Scudamore and Hetmanski (1995) found STC in 8 (17 %) out of 46 samples of poorly stored cereals (wheat, barley and oats). In another study, Scudamore et al. (1997) monitored the occurrence of mycotoxins in grains intended for use as animal feed, again using HPLC, with an LOD of 15 µg/kg. Out of 45 samples of barley and 50 samples of wheat, STC was found in one organically grown wheat sample at a concentration of 18 µg/kg. STC was not detected in any of the 122 samples of cereals (barley, maize, wheat, oats, rice and rye) using HPLC, with an LOD of 3 µg/kg and LOQ of 6 µg/kg

(MAFF, 1998). Negative results for STC were also observed in a survey by the Food Standards Agency (FSA, 2002a), in which a total of 100 samples of rice were monitored.

Using an LC–MS/MS method with an LOD of 0.15 µg/kg and an LOQ of 0.30 µg/kg, Versilovskis et al. (2008a) analysed 95 samples of different Latvian grains from 2006 (wheat, oats, ryes, barley and buckwheat samples) and 120 samples from 2007. STC was found in 14 % of the samples from 2006, with concentrations ranging from 0.7 to 83 µg/kg. In 35 % of the samples from 2007, STC concentrations ranging from 1 to 47 µg/kg were found. The highest concentrations have been detected in wheat and barley, medium concentrations in buckwheat and quite low concentrations in oats and rye samples. In total, for both years 26 % of the 215 analysed samples contained STC above the LOD.

Monbaliu et al. (2010) developed and validated a multi-mycotoxin LC–MS/MS method, accredited according to ISO17025, for the simultaneous detection of 23 mycotoxins, including STC, and used this method for the analysis of feed samples from Belgium. A total of 78 wheat and maize samples taken in 2008, intended for use as animal feed were analysed and STC was not found in any of the samples. Among 367 additional grain samples intended for use as animal feed from the period 2008-2011, 11 samples of wheat, maize and barley showed STC concentrations between 6.9 and 574 µg/kg. Co-occurrence with other mycotoxins was reported for all these 11 STC positive samples (i.e. above 4.75 µg/kg, the decision limit[9]). In three maize samples, co-occurrence of STC (between 7 and 574 µg/kg) with AFB_1 (between 24 and 503 µg/kg) and aflatoxin B_2 (AFB_2) (between 4.3 and 43 µg/kg) was observed (De Saeger, personal communication, 2012).

In Russia, samples of wheat (n = 93), corn (n = 111) and barley (n = 146) intended for use as animal feed were sampled between 2006 and 2009 and analysed using ELISA (LOD/LOQ not reported). The percentage of samples above 100 µg/kg varied depending on the year between 0 and 21 %, 5 and 8 % and 0 and 23 % for wheat, corn and barley, respectively (Kovalenko et al., 2011).

From North America, two reports describe the occurrence of STC in cereal grains using TLC. Scott et al. (1972) analysed 27 grain samples in Canada (wheat, oats, barley and rye) which were associated with lung problems in farmers and elevator operators from farm storage bins. The authors found STC in one (approximately 300 µg/kg) out of 20 wheat samples and no STC was found in the other grains. This was the first published report of STC occurring in an agricultural product. In the United States of America (USA), Stoloff (1976) reported a Food and Drug Administration (FDA) survey of 457 samples of small grains over a two-year period in which STC could not be detected in any of the samples. No recent surveys on the occurrence of STC in North America could be identified.

In Brazil, a total of 130 samples of maize were analysed (10 post-harvest and 120 stored for one year) using TLC, but the toxin was not found in any of the samples. The samples were collected from the region characterised by humid tropical weather, with hot and rainy summers and dry winters (Pozzi et al., 1995). Also in Brazil, Valente Soarez and Rodriguez-Amaya (1989) purchased 60 rice samples at random in retail stores and used a TLC method for the analysis (LOD = 15 µg/kg, LOQ = 35 µg/kg). STC was not detected in any sample.

Several Japanese studies determined STC in brown rice samples. Sugimoto et al. (1977) investigated mycotoxin contamination of mould-infected brown rice during long-term storage in warehouses. They found STC in two samples of contaminated rice with levels of 50 and 450 µg/kg simultaneously with ochratoxin A (OTA) (430 and 230 µg/kg) and citrinin (1130 and 700 µg/kg). Takahashi et al. (1984) used TLC to study brown rice grains, infected by *A. versicolor*, which had been stored in a warehouse for two to three years after harvest. They found that the concentration of STC in the milled rice plus bran fraction was in the range of 3800–4300 µg/kg. However, negative results for STC were obtained by Tanaka et al. (2007), who analysed 48 brown rice samples using HPLC-UV.

[9] If no permitted limit has been established for a substance, the decision limit (CCα) is the lowest concentration level at which a method can discriminate with a statistical certainty of 1-α that the particular analyte is present (Commission Decision 2002/657/EC of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. OJ L 221, 17.8.2002, p. 8-36).

Screening of maize samples used in poultry feed for mycotoxins was carried out in India. Out of 50 samples, three samples were contaminated with STC at concentrations varying from traces to 150 µg/kg (Devi and Polasa, 1982). In another Indian study, wheat, rice and maize samples were collected randomly and screened for different mycotoxins (Pande et al., 1990). Among 30 wheat samples, two were positive for STC with levels of 110 and 145 µg/kg, from 30 rice samples, three were positive with a concentration between 108 and 157 µg/kg and no STC was found in any of the 22 maize samples analysed.

Overall, the available information on the occurrence of STC in grains from the literature is scarce. The EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) summarised the STC occurrence data that were obtained with TLC, HPLC and LC–MS methods. Most of the earlier studies using methods with rather high LODs/LOQs report a high percentage of left-censored data (below the LOD or LOQ) (often 100 %). One recent study using an LC–MS/MS method with an LOD of 0.15 µg/kg and an LOQ of 0.3 µg/kg reported STC-positive samples of grains intended for use as animal feed (26 %).

4.1.2. Occurrence of sterigmatocystin in food

4.1.2.1. Grain-based products

As STC was found in stored grains, it can be assumed that it is present also in grain-based products. Appendix B (Table B2) gives an overview of published occurrence data in grain-based products.

In the UK, Jarvis (1982) investigated 13 maize flour samples and 26 breakfast cereals (six maize based, six oats based and 14 wheat based) and found that only one wheat-based sample was contaminated with STC at a level of 7 µg/kg. A later survey (MAFF, 1998) comprised a total of 128 maize-based food samples, purchased from various retail outlets: breakfast cereals, maize oils, maize on the cob, maize-based thickeners, maize syrup, polenta, tacos, tinned sweet maize, popcorn and maize snacks. STC was determined by HPLC with an LOD of 3 µg/kg. The toxin was not detected in any of the 128 samples analysed.

Stroka et al. (2004) analysed 85 grain-based products purchased from local food markets in northern Italy. The LOD and LOQ of the high-performance thin layer chromatography (HP-TLC) method were 2 and 6 µg/kg for wheat flour, and 4 and 11 µg/kg, respectively, for rice. STC was not detected in any of the 85 samples.

An LC–MS/MS method with an LOD of 0.15 µg/kg and an LOQ of 0.30 µg/kg was used in Latvia for the analysis of 29 samples of different types of bread purchased from the local supermarkets in the city Riga (Versilovskis and Mikelsone, 2008). STC was found in five of the analysed samples at concentrations ranging from 2.4 to 7.1 µg/kg. In total, four out of the five contaminated samples contained whole grains as the main ingredient.

In Brazil, Valente Soarez and Rodriguez-Amaya (1989) purchased 130 samples of maize-based products at random in retail stores and used a TLC method for the analysis (LOD = 15 µg/kg, LOQ = 35 µg/kg). STC was not detected in any sample.

4.1.2.2. Cheese

Cheese is a susceptible product for mould spoilage. *A. versicolor*, a species known to produce STC, is frequently found on hard cheeses that have become infected with this species during production or ripening. The occurrence of STC in cheese has been reported in various publications and was recently reviewed comprehensively by Versilovskis and De Saeger (2010).

In Europe, the first study on the occurrence of STC in cheese was conducted in France, where Lafont et al. (1979) reported the analysis of 235 cheese samples. STC was detected in three contaminated hard cheeses that contained 45, 125 and 330 µg/kg in the outer 2 cm layer. The toxin was not found in

the inner part of the same positive samples. STC was detected also in the outer layer (1–2 cm) of mouldy cheese with concentrations up to 9000 µg/kg in the Netherlands (Northolt et al., 1980; Northolt and van Egmond, 1982) and in Egypt, where 100 samples of hard cheese were analysed using TLC (Abd Alla et al., 1996; Metwally et al., 1997). From the 100 samples, 35 samples contained STC with a mean value of 24 µg/kg and a range between 10 and 63 µg/kg. In Czechoslovakia, among 66 examined samples of hard cheeses taken from local retail shops, three samples were found positive for STC: two samples of Edam cheese contained 7.5 µg/kg and 17.5 µg/kg, one sample of Moravian Block contained 7.5 µg/kg (Bartos and Matyas, 1982). However, several other studies using TLC, HPLC or unreported methods did not detect STC in cheese in a total of 156 samples (Luck et al., 1976; Nowotny et al., 1983; MAFF, 1987, 1998; Lopez-Diaz et al., 1996).

Applying a more sensitive LC–MS/MS method, Versilovskis et al. (2009) analysed (entire) cheeses produced in Latvia (eight samples) and Belgium (13 samples). The LOD and LOQ of the method were 0.03 and 0.1 µg/kg, respectively. STC was quantified in 2 out of 13 Belgian cheese samples with concentrations of 0.52 µg/kg and 1.23 µg/kg. In four of the eight Latvian cheese samples, STC was detected above the LOD, but below the LOQ.

The distribution of STC over surface, rind layer and two cheese layers in six Gouda cheeses (at ages of 2-8 months) as well as the stability of STC in two Gouda cheeses (at ages 6.5 and 8 months) at three different storage temperatures (freezer (–18 °C), refrigerator (+4 °C) and warehouse (+16 °C)), was studied by van Egmond et al. (1982). The authors found that STC contents of the various layers decreased rapidly from the outside to the inner layers. The greater part of *A. versicolor* mycelium and STC were present in the rind layers to a depth not greater than 6 mm. Small amounts of STC could be found in edible parts of the investigated Gouda cheeses. It was uncertain whether STC found in inner layers had been formed in this layer itself or had migrated from the outside into the inner parts. STC was stable for three months at the different temperatures. Earlier, Engel and Teuber (1980) investigated the formation and distribution of STC in cheese after inoculation with *A. versicolor* and *A. nidulans* and found that the toxin was exclusively located near the surface (within 8 mm).

In summary, STC has been detected in the outer layer of mouldy cheese and the available data suggest that contamination of cheese occurs particularly at the surface, following mould infestation during ripening and storage.

4.1.2.3. Nuts

Schroeder and Hein (1977) reported for the first time the occurrence of STC in tree nuts (pecans). They analysed 40 pecan nuts with a TLC method and found one positive sample. The contaminated sample had been purchased from a retail outlet.

In a study of Jiménez et al. (1991), 168 samples of roasted nuts purchased from a Spanish retail market were investigated, including almonds, peanuts, hazelnuts, pistachio nuts, and sunflower seeds. The first three types of nuts were shelled whereas pistachio nuts had usually split shells and sunflower seeds were in-shell. Analysis was done by TLC. STC was not detected in any of the samples. In addition, negative results were obtained in the survey of the Food Standards Agency (FSA, 2002b) where STC was not detected above the limit of quantification (LOQ = 10 µg/kg) in any of the 20 samples of peanuts analysed.

Negative results for STC were also reported from the analysis of 40 peanut samples (El-Maghraby and El-Maraghy, 1987) and 20 hazelnut and 20 walnut samples from Egypt (Abdel-Hafez and Saber, 1993) and a total of 30 samples of six kinds of nuts for human consumption (almond, cashew nut, chestnut, hazelnut, pistachio nut and walnut) from Saudi Arabia (Abdel-Gawad and Zohri, 1993). However, Youssef et al. (2008) found the contamination with STC in a study of untreated, roasted, and roasted and salted peanuts (20 samples of each) prepared for human consumption using TLC. STC was detected in 15 % of roasted nut samples (12.2–16.8 µg/kg) and in 5 % of roasted and salted nut samples (12.2 µg/kg).

4.1.2.4. Other products

Coffee beans

First results on STC in defective and mouldy coffee beans were reported at the 89th Annual Meeting of the Association of Official Analytical Chemists (AOAC) in 1975. No STC was found in any of the 15 samples from 12 different geographical sources (Stoloff, 1976). Several studies determined STC in green coffee beans: Purchase and Pretorius (1973) detected STC in one out of two samples with a concentration of 1 143 µg/kg by TLC in South Africa and De Palo et al. (1977) detected STC in one out of 502 samples in Italy with a concentration of 1200 µg/kg. More recently, STC was found in two out of 30 samples in Saudi Arabia with concentrations of 11 and 13 µg/kg (Bokhari and Aly, 2009). In the latter study a TLC method was used and the sample with the STC concentration of 11 µg/kg was co-contaminated with aflatoxin G_2 (AFG_2) (10 µg/kg).

Fruits and vegetables

Thurm et al. (1979) did not find STC in any of the 134 samples of fresh fruits (grapes, plums, apples, pears, bananas and oranges), sterilised fruits, fruit juices (apple juices, blackcurrant juice and cherry juice), green vegetables (including tomatoes) and sterilised canned vegetables in Germany.

Beer

In a study by Versilovskis et al. (2008b) on 26 Latvian beer samples (9 dark beers and 17 light beers) which were purchased from local supermarkets, HPLC-UV analysis was used with an LOD and LOQ of 0.26 µg/L and 0.68 µg/L, respectively. STC was found in two samples, namely one dark beer with a concentration of 7.8 µg/L and one light beer with a concentration of 4.0 µg/L.

Spices

There are two reports on the occurrence of STC in spices. The first is from India, where 15 different spices (turmeric, coriander, fennel, cinnamon, black pepper, cardamom, greater cardamom, Indian cassia, ammi, cumin, chilli, yellow mustard, Indian mustard, garlic and clove) had been purchased (6 to 9 samples for each spice) from randomly selected major retail outlets (Saxena and Mehrotra, 1989). The samples were not visibly mouldy and were analysed by TLC. STC was found in one out of nine fennel samples (142 µg/kg) and in two out of eight samples of black pepper (105 and 125 µg/kg). The second report is from Egypt, where a total of 24 different spices (5 samples each) were analysed by TLC (El-Kady et al., 1995). STC was detected in the crude extracts of three samples of red pepper (in a concentration ranging from 10 to 23 µg/kg), three samples of caraway (in a concentration ranging from 14 to 18 µg/kg), three samples of cumin (with an average concentration of 11 µg/kg) and one sample of marjoram (with a concentration of 17 µg/kg). In caraway and marjoram, STC co-occurred with AFB_1 and AFB_2.

Other

In Brazil, Valente Soarez and Rodriguez-Amaya (1989) purchased 45 cassava products and 61 dried bean samples at random in retail stores and analysed them by TLC (LOD = 15 µg/kg, LOQ = 35 µg/kg). STC was not detected in any sample.

4.1.2.5. Concluding comments

Overall, the available information on the occurrence of STC in food other than grains from the literature is scarce and often involves old studies applying TLC methods. Considering the low number of available data, the CONTAM Panel summarised the STC occurrence data that were obtained with TLC, HPLC and LC–MS methods. Most of the earlier studies on grain-based products using methods with rather high LODs/LOQs report a high percentage of left-censored data (often 100 %). One recent

study using an LC–MS/MS method with an LOD of 0.15 µg/kg and an LOQ of 0.3 µg/kg reported 5 STC-positive samples of bread out of 29.

Moreover, STC has been occasionally reported to occur in green coffee beans, spices, nuts and beer.

4.1.3. Occurrence of sterigmatocystin in feed

Grain contamination with STC has been reported above. From many of these samples it remains unclear whether or not these grains were intended for human or animal consumption. Only a few studies investigated STC in feedstuff solely and the data are summarised in Appendix B (Table B3).

In the UK, Buckle (1983) reported the analysis of feedstuffs (n = 811), hay (n = 157) and grass silage (n = 451) using TLC and reported one positive hay sample with a concentration below 40 µg/kg. Scudamore et al. (1997) analysed 221 samples of feed ingredients (rice bran, maize gluten and products, cottonseed meal, rapeseed, sunflower meal, olive pulp, palm products, soya, peas/beans and manioc) using HPLC, but STC was not detected.

Scudamore et al. (1998b) tested 40 rice bran samples used in animal feed for the presence of 20 mycotoxins in the UK using HPLC (LOQ = 50 µg/kg). No STC was found in any sample. Scudamore et al. (1998a) analysed STC with a multi-mycotoxin method for 22 toxins in 40 samples of maize gluten (LOD = 100 µg/kg) and in 27 samples of other maize products such as maize germs/brans, baby maize, maize meals, flaked maize and maize screens (LOD = 20 µg/kg). No STC was found in any of these feed samples.

Feedstuffs for dairy cows were tested in 24 dairy farms in the Netherlands. The LOQ for STC was 50 µg/kg. STC was not detected in silage (n = 47), compound feeds (n = 72), ensiled by-products (pressed sugar beet pulp, brewer's grains, potato pulp) (n = 29), forages (fresh grass, dried grass, hay) (n = 13), and feed commodities (rapeseed /soy meal, maize gluten meal, cereals) (n = 8), although other mycotoxins were present (Driehuis et al., 2008a). In another study, Driehuis et al. (2008b) reported that they found no STC in 140 maize silages, 120 grass silages, and 30 wheat silages produced in the Netherlands between 2002 and 2004. An LC–MS/MS method was used with an LOQ of 50 µg/kg.

Monbaliu et al. (2010) used a multi-mycotoxin LC–MS/MS method, accredited according to ISO17025, for the simultaneous detection of 23 mycotoxins and analysed four sow feed samples taken in 2008. All samples were contaminated with at least one mycotoxin, but STC was not found in any of the samples. Among 282 additional feed samples from the period 2008–2011, five mixed feed samples and three samples of maize silage showed STC concentrations between 8.6 and 17.2 µg/kg. Co-occurrence with other mycotoxins was reported for all STC-positive samples (i.e. above 4.75 µg/kg, the decision limit[9]) (De Saeger, personal communication, 2012).

In Russia, samples of soybean (n = 15), sunflower cake (n = 53), mixed feed for piglets 0-2 months old (n = 120), mixed feed for piglets 2-4 months old (n = 94), mixed feed for sows and fattening pigs (n = 64), 'dry milk, whole milk substitute, protein-mineral-vitamin supplement' (n = 28), peas (n = 22) and bran (n = 18) were sampled between 2006 and 2009 and analysed using ELISA (LOD/LOQ not reported). The percentage of samples above 100 µg/kg varied depending on the year between 0 and 50 %, 0 and 22 %, 3 and 18 %, 0 and 2 %, 0 and 8 % and 0 and 14 %, for soybean, sunflower cake, mixed feed for piglets 0-2 months old, mixed feed for piglets 2-4 months old, mixed feed for sows and fattening pigs and 'dry milk, whole milk substitute, protein-mineral-vitamin supplement', respectively. No samples exceeded 100 µg/kg for peas and bran (Kovalenko et al., 2011).

El-Shanawany et al. (2005) tested 40 Egyptian silage samples for mycotoxins using TLC and found STC in two samples.

Vesonder and Horn (1985) reported the occurrence of 7750 µg STC/kg dairy cattle feed that was infected by *A. versicolor* in the USA in 1983. The feed consisted of maize, cottonseed and a protein mix.

Overall, the available information in the literature on the occurrence of STC in feed other than grains is scarce. Most of the studies report a high percentage of left-censored data (often 100 %). However, these analyses were mostly conducted with methods with rather high LOD/LOQs. A recent study using an LC–MS/MS method reported 3 % of STC-positive samples of feed.

4.2. Occurrence of sterigmatocystin in food and feed: call for data

In October 2010, EFSA launched a call for data on mycotoxins and phytotoxins, including STC, in food and feed. EFSA collected and evaluated the results reported from the analysis of 581 samples, including 247 food and 334 feed samples. It cannot be excluded that samples described in Section 4.1 were also submitted to EFSA during this call for data.

The data submission to EFSA followed the requirements of the EFSA Guidance on Standard Sample Description for food and feed (EFSA, 2010a). SAS Enterprise software was used to process the submitted data.

4.2.1. Summary of data collected

The origin of the 581 food and feed samples reported from three European countries is illustrated in Figure 2. The United Kingdom and Czech Republic provided, respectively, 53 % and 47 % of the analytical results on food, whereas the Netherlands and Czech Republic provided, respectively, 81 % and 19 % of the analytical results on feed (Figure 2).

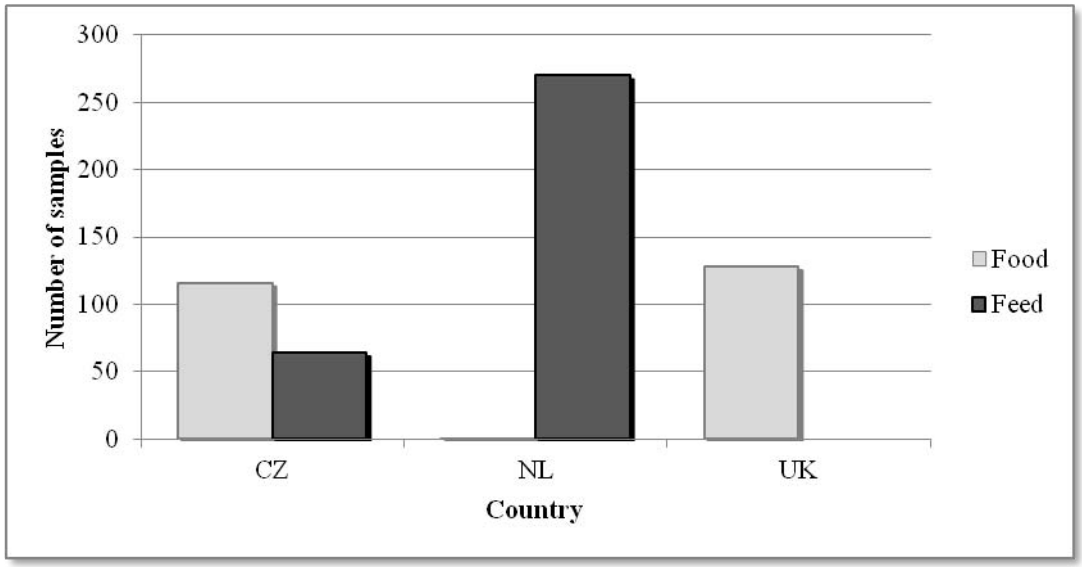


Figure 2: Distribution of food and feed samples for which sterigmatocystin concentrations were reported to EFSA across European countries (CZ: Czech Republic; NL: the Netherlands; UK: United Kingdom).

The distribution of analytical results over the sampling years is illustrated in Figure 3. Almost 60 % of the analytical results on food were obtained in 2009 and 2010 while the remaining 40 % of the results were obtained in 2000. The results on feed samples were from 2003 (81 %) and 2010 (19 %).

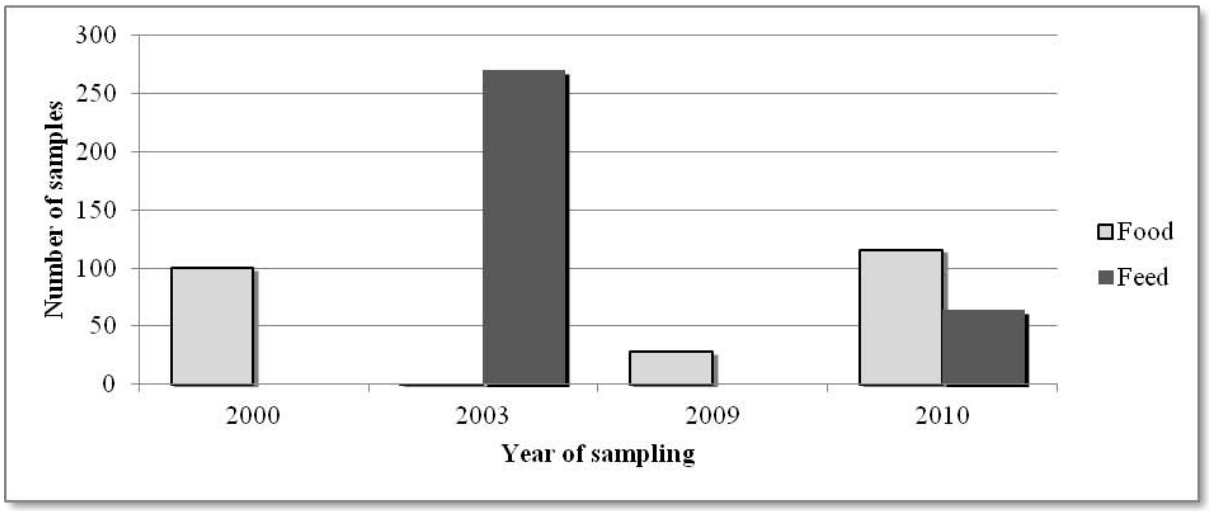


Figure 3: Distribution of food and feed samples for which sterigmatocystin concentrations were reported to EFSA over the sampling years.

4.2.2. Occurrence data from food samples: data analysis

Data were classified according to FoodEx, which is a food classification system developed by EFSA's Dietary Chemical Monitoring Unit in 2009 with the objective to simplify the link between occurrence and food consumption data when assessing the dietary exposure to hazardous substances (EFSA, 2011a). FoodEx contains 20 main food groups (first level), which are further divided into subgroups having 160 items at the second level, 1 261 items at the third level and about 1 800 endpoints (food names or generic food names) at the fourth level.

Data on STC in food were mainly reported in the food group 'Grains and grain-based products' and only four samples were reported in the food group 'Snacks, desserts, and other foods'. Within the food group 'Grains and grain-based products', bread and rolls represented almost 50 % of the results, followed by rice (40 %), grain milling products (6 %) and fewer than five samples for the remaining sub-groups (see Appendix C, Table C1).

The analytical results on STC in food were obtained by LC–MS/MS (n = 117) or HPLC coupled with FLD (n = 130).

The detection capabilities varied with the analytical techniques, the food matrices and the analysing laboratories. In the case of HPLC-FLD methods, the LOD varied from 2 to 3 µg/kg whole weight (median of 3 µg/kg whole weight), whereas for LC–MS/MS, the LOD was 5 µg/kg whole weight. The LOQs for the two analytical methods were reported as twice the LODs.

All analytical results on STC in food were reported as either < LOD (47 %) or < LOQ (53 %). Therefore, in accordance with international guidance no dietary exposure assessment could be performed based on this data set (GEMS/Food-EURO, 1995; WHO, 2009; EFSA, 2010b) and the CONTAM Panel concluded that the data are too limited to carry out a reliable human dietary exposure assessment.

4.2.3. Occurrence data from feed samples: data analysis

Feed was classified according to the catalogue of feed materials specified in the Commission Regulation (EU) No 575/2011[10] of 16 June 2011 creating the Catalogue of feed materials. Most of the results were reported for 'Forages and roughage, and products derived thereof' (n = 130, 39 %) and 'Cereal grains, their products and by-products' (n = 123, 37 %) (Appendix C, Table C2). All results were obtained by LC–MS/MS with an LOD equal to 5 µg/kg. The LOQ was reported only for 19 % of the data and was equal to 10 µg/kg. All left-censored data in feed were reported as < LOD. All analytical results were reported as whole weight.

Only four quantified results were reported in feed: two of unspecified forage and roughage (8 and 8.9 µg/kg), one sample of lucerne pellets (28 µg/kg) and one sample of cocoa hulls (14 µg/kg). Therefore, the CONTAM Panel concluded that the data are too limited to carry out a reliable animal dietary exposure assessment.

4.3. Food and feed processing

4.3.1. Food processing

Several studies reported on the changes in STC content in food during food processing such as milling, bread and cheese making and roasting.

Takahashi et al. (1984) reported that most of STC in brown rice was removed in the milling process; however, it was not possible to remove the toxin completely.

Versilovskis and Bartkevics (2012) tested the stability of STC during bread making using a contaminated batch of wheat with an STC concentration of 83 ± 8 µg/kg. The flour obtained from wheat grains was used for whole-grain bread baking for 17 minutes at 200–220 °C. The mean content of STC in baked bread was 48 µg/kg, which was equivalent to 78 µg/kg in grains. Statistically there was no significant difference between the results obtained in contaminated grains and the results obtained in bread baked from these grains and as such STC was found to be stable during the bread making process.

Abd Alla (1994) added STC to milk and used this contaminated milk for cheese making. It was shown that the disappearance of STC from contaminated milk depends on the type of starter culture, the storage period and the type of milk. The same group showed also that during cheese processing, 80 % of the toxin present in milk was found in the curd and only 20 % was found in the whey, related to low solubility in aqueous media, according to the authors (Abd Alla et al., 1996; Metwally et al., 1997).

The influence of roasting on the STC content was investigated by Bokhari and Aly (2009), who roasted coffee beans at 200 °C for 6–15 minutes using an electric oven. After 6 or 8 minutes, no decrease in STC content was found. Increasing the roasting time to 10–12 minutes reduced the STC concentration by 40 to 50 %, and after 15 minutes the STC concentration was reduced by 70 %. These data confirm earlier results reported by Stoloff (1976) on roasting at about the same temperature for 20 minutes.

In conclusion, food processing can result in a decrease of the STC concentration; however, the extent depends on the type of food and the processing conditions.

4.3.2. Feed processing

No data could be identified on the fate of STC during feed processing. There is a lack of knowledge on the effects of grinding, milling, pelleting, steam conditioning, extrusion, expelling and micronisation, and other processes used in feed processing on STC concentrations.

[10] Commission Regulation (EU) No 575/2011 of 16 June 2011 on the Catalogue of feed materials. OJ L 159, 17.6.2011, p. 25-65.

5. Food and feed consumption

No food or feed consumption data were used in this opinion because no exposure assessment was possible.

6. Exposure assessment in humans and animals

6.1. Human exposure assessment of sterigmatocystin

6.1.1. Previously reported human exposure assessments

No published human dietary exposure assessments could be identified.

6.1.2. Dietary exposure to sterigmatocystin

Only limited STC occurrence data in food were submitted to EFSA by two European countries. All of the data were left-censored and only two food groups were covered. Therefore, the Panel concluded that these data are too limited to carry out a reliable human dietary exposure assessment.

6.2. Dietary exposure assessment of sterigmatocystin in animals

Only limited occurrence data in feed were submitted to EFSA by two European countries and 99 % of the data were left-censored. Therefore, the Panel concluded that these data are too limited to carry out a reliable animal dietary exposure assessment.

6.3. Non-dietary exposure

A. versicolor prevalent in the indoor environment can produce STC up to 1 % of its biomass (Nielsen, 2003). *A. versicolor* is able to grow on very nutrient-poor materials such as concrete and plaster. STC and 11-methoxy-STC[11] were detected by HPLC-DAD and TLC with $AlCl_3$ staining, in each of five isolates of *A. versicolor* artificially inoculated on pine wood, wallpaper, gypsum board and chipboard (Nielsen et al., 1998). The concentrations of STC varied and could reach up to 23 µg/cm^2 in wallpaper samples. This is of concern in water-damaged buildings as large quantities of STC on the building materials can be present.

Sample collection is a key step in the analysis of mycotoxins in buildings, but no harmonised sampling protocol exist. For the assessment of human exposure, only the mycotoxin-containing particles (including fungal conidia) that can become airborne are of interest. Therefore, a combination of surface, dust and air sampling is recommended (Nielsen, 2003; Polizzi et al., 2009).

STC was detected in 2 out of 11 carpet dust samples collected from eight dwellings in northern Germany with visible mould growth and/or signs of indoor dampness. The analysis was done by means of LC–MS/MS and the concentrations ranged between 2 and 4 ng/g of dust (Engelhart et al., 2002).

Seventy-nine bulk samples of mouldy interior surfaces from Finnish buildings with moisture problems were analysed for 17 mycotoxins by means of LC–MS/MS. Collected building materials included wallpaper, cardboard, wood, plywood, plasterboard, paper-covered gypsum board, mineral wool, plaster, sand, soil, linoleum, polyurethane insulation, pipe insulation and paint. The mycotoxin analysis revealed that STC was the most prevalent mycotoxin in the buildings. It was detected in 19 samples (24 %) and in concentrations up to 31 000 ng/g. *A. versicolor* was present in most STC-containing samples (Tuomi et al., 2000).

The presence of STC in indoor environments was also confirmed by Bloom et al. (2007). Using LC–MS/MS, they could detect STC in settled dust from water-damaged buildings and homes. The mycotoxin was found in one out of eight samples at a concentration of 17 ng/g. It was also found in

[11] Reported by the authors as 5-methoxy-STC.

25 of the 62 building materials in the range from 1.9 to 1 100 ng/g, and usually it co-occurred with two or more other mycotoxins. A follow-up study by Bloom et al. (2009a) in water-damaged indoor environments in Sweden showed that STC was the third most frequently found mycotoxin in building materials (22 %) and the most common in cultures of airborne fungal particles (41 %). STC was detected in building material samples in the concentration range of 4.9–150 000 ng/g and in cultures in the range of 3.6–10 900 pg/cm^2. Settled dust was found to be free of STC.

Another investigation carried out by Polizzi et al. (2009) in seven water-damaged buildings in Belgium revealed the presence of one or more mycotoxins in 62 out of the 99 samples analysed by means of multi-mycotoxin LC–MS/MS analysis (Delmulle et al., 2006). The mycotoxins found were mainly roquefortine C, chaetoglobosin A and STC, but roridin E, OTA, AFB_1 and AFB_2 were also detected. STC was present in air samples (0.0034–1.77 ng/m^3) and in dust, wallpaper, and scraped mycelium (0.063–778 ng/cm^2).

Ventilation systems of Finnish schools were examined for the presence of mycotoxins including STC. Only in one case was a small quantity of this toxin detected (2.7 pg/cm^2) in dust collected by wiping the supply air handling unit; however other mycotoxins including but not limited to beauvericin and verrucarol were also present (Hintikka et al., 2009).

Bloom et al. (2009b) collected dust samples in New Orleans dwellings. The houses were contaminated with moulds because of the flooding after hurricane Katrina. The collected samples were analysed for the presence of mycotoxins by LC–MS/MS. STC was detected in two samples at concentrations of 16 and 28 ng/g dust. Additionally, *Aspergillus* spp. were identified in these two samples.

Vishwanath et al. (2011) focused on two relatively different indoor environments—inhabited houses and waste management units. The study was intended to estimate exposure to contaminants (including mycotoxins) through ingestion and/or inhalation of indoor dust. The samples of settled floor dust were collected using a vacuum cleaner and analysed by LC–MS/MS. STC was detected in both environments at concentrations not higher than 45 ng/g in the waste units and 11 ng/g in the houses.

It should be noted that the above-mentioned studies and contamination data, result from targeted sampling from buildings with clear visible mould growth and/or signs of indoor dampness or water damage. Although it is at this stage impossible to quantify human STC exposure from indoor environments, inhalation might lead to additional STC exposure.

7. Hazard identification and characterisation

7.1. Toxicokinetics

7.1.1. Absorption

The absorption of STC was studied in a single vervet monkey (*Chlorocebus pygerythrus*) given a single oral dose of 100 mg ^{14}C-labelled STC (18.4 mg/kg b.w., purity 99.5 %, 0.145 mCi) (Steyn and Thiel, 1976). The maximal rate of absorption from the gastrointestinal system was not higher than 30 % of the applied dose. In Sprague-Dawley rats, peak plasma levels were reached after 30 minutes following an oral bolus administration of 8 mg/kg b.w. of ^{14}C-STC (Walkow et al, 1985; details of the study are described below in Section 7.1.2). In this study the rate of absorption was not calculated, but plasma concentration-time curves showed significant differences between female and male rats in both age groups tested. The cumulative renal excretion varied between 7.3 and 10.3 % of the administered dose, suggesting a limited rate of absorption also in rats.

7.1.2. Distribution

Kinetics of STC were studied by Wang et al. (1991) in 30 male Wistar rats orally treated with ^{3}H-STC (0.5 mCi/kg b.w. radioactive purity 95 %). The highest concentration of radioactivity in serum appeared three hours after treatment and the half-life was calculated to be 30 minutes. Radioactivity

was 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. An initially high accumulation of radioactivity was also found in stomach, which is in accordance with acanthomatous changes seen in rats chronically exposed to STC (Purchase and van der Watt, 1970a) and gastric erosions observed in Mongolian gerbils treated orally with STC (Kusunoki et al., 2011).

In a repeated dose study, male and female immature and mature Sprague–Dawley rats (n = 5 per group per sex and age group) were exposed to STC (8 mg/kg feed equally divided over 13 days). On the 14th day the rats received an oral bolus administration of 8 mg/kg b.w. of ^{14}C-STC (unknown purity) dissolved in wheat germ oil. 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 (Walkow et al., 1985).

7.1.3. Metabolism

Data on the biotransformation of STC are scarce. The few studies published to date indicate that phase I metabolism of STC comprises cytochrome P450 (CYP)-mediated formation of a reactive epoxide as well as mono- and dihydroxylation reactions. As phase II metabolites, a glucuronide of STC and of monohydroxy-STC have been reported, together with a sulphate conjugate of monohydroxy-STC and a glutathione (GSH) adduct of a monooxygenated STC. However, the structure of most of these metabolites is not completely identified and, in part, controversial, as outlined below.

7.1.3.1. Phase I metabolites

The most conclusive evidence for the formation of a reactive STC epoxide was obtained by Essigmann et al. (1979), when STC was incubated under cell-free conditions with DNA in the presence of rat liver microsomes. Subsequent isolation and degradation of the DNA followed by HPLC analysis revealed the presence of a covalent adduct, which was purified and identified by nuclear magnetic resonance spectroscopy and mass spectrometry as 1,2-dihydro-2-(N^7-guanyl)-1-hydroxy-STC (see also Section 7.2.5). The same DNA adduct was formed when isolated rat liver was perfused with STC (Essigmann et al., 1980). From the chemical structure and stereochemistry of this adduct, it was concluded that an exo-STC-1,2-oxide, i.e. an epoxide of STC with the epoxide ring in trans position to the bulky rest of the molecule, was the metabolite that reacted with DNA.

In a recent study using recombinant human CYP1A1, 1A2, 2A6, 2A13, and 3A4, the *in vitro* metabolism of STC was investigated (Cabaret et al., 2010, 2011). O-Demethylation of STC was not observed with any of the five CYP isoforms. Three metabolites (M1, M2, M3) were detected by LC-MS with electrospray ionization and tentatively identified by their mass spectra. To M1, the structure of 12c-hydroxy-STC was assigned. M2 was proposed to represent 1,2-dihydroxy-STC, possibly resulting from the hydrolysis of STC-1,2-oxide. M3 was suggested to be the glutathione (GSH) adduct of STC-1,2-oxide. However, these structure assignments, particularly of M2 and M3, were questioned by Krol (2011), mostly because of discrepancies between the mass spectra and the proposed structures. As alternative metabolic pathways, which would be in better agreement with the mass spectra obtained by Cabaret et al. (2010, 2011), Krol (2011) suggested hydroxylation of STC at positions 9 and/or 11 (see Figure 1). This would lead to catechols/hydroquinones and through subsequent oxidation to electrophilic quinones capable of forming GSH adducts. However, this aromatic hydroxylation of STC is still not confirmed, and the exact chemical structures of M1, M2, and M3 have yet to be elucidated. It should be noted that chemical syntheses of STC-1,2-oxide, its hydrolysis product STC-1,2-dihydrodiol and its GSH conjugate 1-hydroxy-2-(S-glutathionyl)-1,2-dihydro-STC have been reported (Baertschi et al., 1989; Raney et al., 1992), but this information has not yet been used for the rigorous identification of STC metabolites.

The five human CYP isoforms used by Cabaret et al. (2010) differed in their ability to oxidize STC. In the absence of GSH, M1 was only generated by CYP1A1 and 1A2, and M2 only by CYP1A1. In the presence of GSH, M3 was only formed by CYP1A2 and 3A4. Evidence for the ability of individual

CYP isoforms to metabolically activate STC is available from several genotoxicity tests. For example, STC was more efficiently activated by human CYP3A4 than by CYP3A5 to DNA-damaging products in two *Salmonella typhimurium* tester strains (Yamazaki et al., 1995). In the same test system, experiments with lung microsomes of rats treated with various CYP inducers suggested that CYPs other than those of the CYP1A family activate STC (Shimada et al., 1992). In contrast, rat liver CYP1A1, 1A2 and 2B1 stably expressed in immortal Chinese hamster V79 cells activated STC to a potent clastogen as measured by the induction of micronuclei containing chromosome fragments, but not whole chromosomes (Ellard and Parry, 1993). Shimada et al. (1996) showed that human CYP1B1, which is widely expressed in extrahepatic tissues, does not activate STC in two *S. typhimurium* tester strains. However, an increased mutation frequency of STC was reported in *Saccharomyces cerevisiae* after transfection of rat CYP2B1 (Black et al., 1992). The capacity to activate STC to cytotoxic metabolites was essentially similar for human fetus-specific CYP3A7 and adult CYP3A4 stably expressed in Chinese hamster lung cells (Hashimoto et al., 1995).

7.1.3.2. Phase II metabolites

The first identified conjugate of STC was its glucuronide, isolated as a major metabolite from the urine and bile of a vervet monkey after oral administration of ^{14}C-labelled STC (Thiel and Steyn, 1973; Steyn and Thiel, 1976). This metabolite released STC upon hydrolysis with ß-glucuronidase, showing that it was the glucuronide of parent STC and not of a hydroxylation product. STC has only one functional group suitable for glucuronidation, i.e. the hydroxyl group at C-8 (Figure 1). When the urine of rats after intraperitoneal (i.p.) injection of STC was analysed for metabolites using an immunosorbent method with polyclonal antibodies, STC glucuronide was the only conjugate observed (Olson and Chu, 1993a). Likewise, when STC was incubated with cultured primary epithelial cells from the trachea of pigs, STC-8-O-glucuronide was the only conjugated metabolite detected by LC-ESI-MS (Cabaret et al., 2010, 2011). After pre-treatment of the cells with the CYP inducer ß-naphthoflavone, two additional conjugates were observed by the same authors and tentatively identified as monoglucuronide and monosulfate of a monohydroxylated STC. The exact chemical structures of these conjugates are as yet unknown. For a discussion of the GSH conjugate, see Section 7.1.3.1.

7.1.4. Excretion

The study of Walkow et al. (1985) showed that most of the orally given STC in Sprague-Dawley rats was eliminated in the faeces (64–92 %), and about 10 % via urine. The calculated elimination half-life in these Sprague-Dawley rats varied between 61.5 h in immature females and 130 h in mature male rats and the cumulative total elimination rate (urine and faeces) after 96 h exceeded 99 % in immature and mature males, and varied between 71.5 and 77.4 % in immature and mature females, respectively. The plasma concentration–time curves provided evidence of entero-hepatic recirculation. In contrast, Olson and Chu (1993b) found in Fischer rats treated i.p. with 1, 4, 8 and 16 mg STC/kg b.w. (n = 4 per group) that, within 24 hours, only 3.1 % of the administered dose was recovered in urine. This apparent difference from the study of Walkow et al. (1985) is probably due to the lack of appropriate standards in the ELISA method used by Olson and Chu (1993b), which might not detect conjugated STC.

Urinary and biliary excretion of STC was also studied in one vervet monkey given orally ^{14}C-STC (Thiel and Steyn, 1973; Steyn and Thiel, 1976). The major fraction (50–80 %) of the oral dose was recovered in faeces within 48 hours.

7.1.5. Carryover into animal products

The carryover of STC from feed to milk was investigated in two cows exposed via feed to approximately 5 to 10 mg STC per day for two weeks (van Egmond et al., 1978). No STC could be detected in milk (LOD of 1 µg STC/kg milk); based on this LOD the authors calculated that less than 0.4 % of the STC was transferred to milk. As TLC was applied as the method of detection for STC residues in milk, potential metabolites of STC could not be identified. The CONTAM Panel concluded

that there are insufficient data to assess the rate of carryover of STC into milk. No information about the carryover of STC and/or its metabolites into animal products such as meat and eggs was identified.

7.1.6. Concluding comments

The scarce information available suggests that absorption of STC is limited following oral exposure. STC is metabolised in the liver and lung by various cytochrome P450 enzymes into different hydroxymetabolites and its reactive exo-epoxide that readily forms DNA adducts. Excretion of both conjugated parent STC and its hydroxylated metabolites occurs via bile and urine.

7.2. Toxicity in experimental animals

7.2.1. Acute toxicity

LD_{50} values of STC in experimental mammals as reported in the literature are summarized in Table 2. STC has relatively low acute oral toxicity. Although STC is structurally related to AFB_1, STC's acute toxicity is 10 or more times lower: Butler (1964) reported in male rats that the i.p. LD_{50} values for STC and AFB_1 are 60 and 6 mg/kg b.w., respectively, and that the per os (p.o.) LD_{50} values are 166 and 7.2 mg/kg b.w., respectively. Wong and Hsieh (1980) reported for AFB_1 a p.o. LD_{50} in rats ranging from 5.5 to 17.9 mg/kg b.w. The accuracy of the LD_{50} values presented in Table 2 is questionable, since large volumes of solvent were used in order to overcome the problem of low solubility of STC without quantifying the influence of the volume of solvent and the unabsorbed STC on the oral LD_{50} (Purchase and van der Watt, 1969).

Table 2: LD_{50} values of sterigmatocystin in experimental rats and monkeys as reported in the literature.

Animal	Sex	Solvent	Route	LD_{50} (mg/kg b.w.)	95 % CI	Reference
Rat	M	Dimethylformamide	p.o.	166	113–224	Purchase and van der Watt (1969)
Rat	M	Dimethylformamide	i.p.	60	46–77	Purchase and van der Watt (1969)
Rat	F	Wheat germ oil	p.o.	120	92–155	Purchase and van der Watt (1969)
Rat	M	Wheat germ oil	i.p.	65	37–109	Purchase and van der Watt (1969)
Monkey	M	Dimethylsulphoxide	i.p.	32	15–70	Purchase and van der Watt (1970b)

M: male; F: female; p.o.: per os; i.p.: intraperitoneal; b.w.: body weight; CI: confidence interval.

The target organs of acute STC toxicity are liver and kidney. In rats treated with high doses, areas of necrosis in the liver and kidney were seen (Purchase and van der Watt, 1969). In the liver, STC causes degenerative changes and necrosis depending on the route of administration. In i.p.-treated animals, liver injuries are seen first in the periportal zone, while in orally treated animals lesions are localised around the central vein and are milder than when the same dose is applied i.p. Histopathological changes after administration of doses lower than the LD_{50} are focal necrosis affecting single cells or groups of cells together with the enlargement of some nuclei and the appearance of multiple nucleoli. At STC doses higher than the LD_{50} (150–200 mg/kg b.w., 95 % purity), severe periportal necrosis with haemorrhage in necrotic areas was described. In animals treated i.p. with high doses of STC, the peritonitis was so severe that it could have contributed to death. In the kidney, at doses between 10 and 100 mg/kg b.w., STC causes cortical haemorrhage, hyaline casts in the collecting tubules and pyknotic nuclei in the tubular cells at the cortico-medullary junctions. There was also hyaline degeneration or necrosis of tubular epithelial cells. At doses in the range of 100–144 mg/kg b.w., lesions were accompanied by degeneration and necrosis of glomeruli with hyaline thickening of the basement membrane. The most prominent findings at doses above 144 mg STC/kg b.w. were massive haemorrhage and necrosis. The degree of kidney damage was independent of the vehicle used to dissolve STC.

Young animals are apparently more sensitive to STC toxicity than adults. A single s.c. injection of 0.03 mL of 1 % gelatine suspension with 100, 50, 10, 5, 1 and 0.5 mg STC/kg b.w. was given to the

progeny of nine dams of CD2F mice within 24 hours after birth (Fujii et al., 1976). Most of the young mice died within five days after treatment. In the three highest dose groups almost all animals died.

In 40-hour-old chicken embryos, 1.0 µg STC/egg caused 100 % mortality during the 24-hour observation period (Vesely et al., 1982). When the same dose was given to five-day-old chicken embryos, there was no mortality, but all embryos died at a dose of 10 µg of STC/egg (Schroeder and Kelton, 1975). Another study reported an LD_{50} value for five-day-old chicken embryos of 14.9 µg per egg (Terao, 1983).

Considering exposure of STC via the indoor environment, a recent study describes the effect of an intratracheally instilled single STC dose of 13 mg/kg lung weight on inflammation-associated gene transcription in lung tissue in pathogen-free white Swiss Webster mice sacrificed 4 or 12 hours afterwards (Miller et al., 2010). The results were compared with those of the control group instilled with physiological solution (five animals per group). There were no histological changes seen in animals sacrificed four hours after STC treatment compared with controls, but in animals sacrificed 12 hours after treatment mucus production was abundant, and bronchiolar and alveolar spaces and epithelium showed signs of inflammation with irregular thickened epithelium, mucus-like debris and alveolar walls that appeared oedematous. In animals sacrificed four hours after STC treatment, reverse-transcription real-time polymerase chain reaction revealed the modulation of the inflammation-associated genes that was not so prominent 12 hours after treatment.

In conclusion, the acute oral toxicity of STC is relatively low (range 120–166 mg/kg b.w.). Liver and kidneys are the target organs of acute toxicity. Hyaline degeneration was observed in the kidney and necrosis and haemorrhages were observed in the liver and in the kidneys. Following inhalation (intratracheal installation) of STC, a non-specific but severe inflammatory response of the lung tissue was observed.

7.2.2. Repeated dose studies and subchronic toxicity

Considering the previously observed hepatotoxicity of STC, van der Watt and Purchase (1970) studied the sequence of the timely appearance of liver lesions in male weanling Wistar rats (initial weight 50 g; n = 16) exposed to the high concentration of 100 mg STC/kg feed (purity 95 %) for 16 weeks and sacrificed in pairs every other week starting after two weeks of exposure. After an exposure of two weeks, single cell necrosis and marked variations in the size of both nuclei and cells were observed, as well as few necrotic foci without inflammation and prominent Kupffer cell proliferation. After 12 weeks of exposure, foci enlarged and were macroscopically visible and progressed into large hyperplastic nodules surrounded by degenerating and necrotic hepatocytes (Purchase and van der Watt, 1970b). In contrast to the observation of liver lesions following exposure to AFs, there was no massive bile duct proliferation observed after STC treatment. With continued feeding, first hepatic neoplasm was observed at 40 weeks, and at 50 weeks all surviving animals developed HCC.

In a study with repeated intragastric dosing of STC (20 mg/kg b.w. every 14 days for 12 months, number of animals not reported) in vervet monkeys, histological liver changes were ascertained by liver biopsies performed at nine-week intervals for a period of 12 months (Purchase and van der Watt, 1970b). After four to six months, the treatment caused icterus and chronic hepatitis. Continuing the treatment resulted in aggressive hepatitis with single-cell necrosis of hepatocytes, progressive growth of the fibrous septa and disruption of the lobular architecture. After 12 months' exposure, large hyperplastic nodules, containing hepatocytes with pleomorphic nuclei, were observed throughout the liver.

In guinea pigs (n = 10), oral treatment with STC (4.2 mg/animal for two weeks) caused diffuse fatty degeneration of hepatocytes and focal necrosis without any particular lobular distribution (Richard et al., 1978). In the necrotic foci, a proliferation of Kupffer cells and neutrophilic infiltration were seen.

7.2.3. Reproductive and developmental toxicity

Only one screening study (Schroeder and Kelton, 1975) on developmental toxicity was identified. STC was given to five-day-old chick embryos in the yolk sac of eggs at a dose of 5–7 µg/egg. Twisted feet were observed in the treated animals. The authors considered this finding as inconclusive as this condition may occur spontaneously. No further studies on reproductive and developmental toxicity of STC could be identified.

7.2.4. Immunotoxicity

In an early study carried out in 1978 (Richard et al., 1978) it was shown that STC, given orally to guinea pigs for two weeks (4.2 mg/animal), induced a significant reduction of complement activity. Even if there was no significant effect on the total serum protein values in guinea pig serum, effects on α2- and β- globulins, which are a major component of complement, were noted. Apart from these effects, also significant weight loss and hepatotoxicity was observed. As components of complement are produced in the liver, it can therefore not be concluded that STC has a specific effect on complement, or is specifically immunotoxic.

Zhang et al. (2012) treated specific pathogen free Balb/c mice (n = 32) i.p. with STC dissolved in dimethylsulphoxide (DMSO)/saline at a dose of 3 mg/kg b.w. It was observed that tumour necrosis factor-alpha (TNF-α) levels as well as interleukin (IL)-12p35 levels were decreased in treated animals, whereas IL-6 levels were transiently increased in peripheral blood mononuclear cells (PBMCs) isolated from these animals. Concomitant analyses with peritoneal macrophages isolated from the same animals showed a significant decrease in the levels of all three cytokines, TNF-α, IL-6 and IL-12p35. The severity of these effects increased with time post administration (measured up to 24 h after treatment). The authors comment that such an impairment of the host defence system is comparable to the immunosuppressive effects discussed, for example, for trichothecenes (T-2 toxin), gliotoxin and other immunosuppressive mycotoxins. However, only one dose of STC was applied, hence no clear dose-response relationship could be assessed. Moreover, the study does not provide any information on effects of the treatment on any other possible target of STC toxicity, which would have been helpful in concluding on potential specific immunotoxicity of STC.

Liu et al. (2012) showed that 24 h after a single i.p. STC administration to BALB/c mice (3, 30, 300 and 3000 µg/kg b.w.), the percentage of FoxP3+ regulatory T cells (as well as the FoxP3 expression level) are significantly increased in PBMCs, the spleen and the thymus (mainly for treatments ≥ 30 µg/kg b.w.). Regulatory T cells expressing FoxP3 play a key role in immune homeostasis by suppressing immune responses of a variety of immune cells (Schmidt et al., 2012). Increases in regulatory T cells can result in suppressing an effective immune surveillance of carcinogen-induced tumours (Betts et al., 2007). The study by Liu et al. (2012) also reported a statistically significant decrease of plasmacytoid dendritic cells (pDCs) in the thymus and a significant increase of this cell population in the spleen at doses of 30, 300 and 3000 µg/kg b.w. pDCs populations in the thymus are involved in the induction of regulatory T cells (Hanabuchi et al., 2010; Martin-Gayo et al., 2010) but the overall effect of STC on pDC function remains to be elucidated. It should be noted that the study addresses effects after i.p. injection of STC at high concentrations and that there is no information provided on other effects of the treatment on any other possible target of STC, and this hinders the interpretation of these findings as specific immunotoxic effects.

A series of papers on *in vitro* experiments with human peripheral blood lymphocytes and murine peritoneal macrophages showed that STC inhibited the expression and secretion of IL-12 (Xing et al. (2005) and Huang et al. (2002), cited in Zhang et al., 2012) and affected IL-2, interferon-γ and IL-4 expression and secretion in murine spleen cells (Xing et al. (2005), cited in Zhang et al., 2012). In addition, Xing et al. (2005) demonstrated that STC reduced the expression of human leukocyte antigen (HLA) class I at the level of both RNA and protein. HLA-class I antigens have been associated with numerous immunological reactions and autoimmune diseases as well as with cellular apoptosis and development of neoplastic diseases, and the findings that STC can reduce HLA-1 expression suggest the immunomodulatory effects of STC. It should be noted, however, that these results were obtained

with rather high concentrations ranging between 0.5 and 2 mg/L, and that the relevance of these findings for the *in vivo* exposure remains to be elucidated.

In conclusion, the results from *in vivo* and *in vitro* studies suggest that STC may have immunomodulatory activity. However, firm conclusions cannot be drawn, as the *in vivo* data were difficult to interpret as specific immunotoxic effects. Moreover, the relevance of the *in vitro* data were difficult to interpret as these experiments were conducted with rather high concentrations of STC.

7.2.5. Genotoxicity

Genotoxicity of STC has been tested in bacteria and mammalian cells *in vitro* and in rats *ex vivo* and *in vivo*. It was tested very often together or in comparison with other mycotoxins, especially with AFB_1.

In a large screening of 300 carcinogens and non-carcinogens by the standard *S. typhimurium* assay in the presence of rodent liver S9 mix, STC was approximately 10 times less mutagenic than AFB_1 on a molar basis in the tester strains TA100, TA98 and TA1538 (McCann et al., 1975). The mutagenicity of STC in *S. typhimurium* was later confirmed in several studies by using a variety of tester strains in the presence of rodent or human liver microsomes while the comparison with AFB_1 did not give consistent results (Tang and Friedman, 1977; Kuczuk et al., 1978; Ueno et al., 1978; Wehner et al., 1978; Mori et al., 1986). The report of McCann et al. (1975) implied that STC has similarly lower activities in base-pair and frame-shift mutagenesis as compared to AFB_1. Mori et al. (1986) confirmed a lower activity of STC for frame-shift mutations but showed higher levels of induced base-pair mutations as compared to AFB_1. STC was also positive in the *rec* assay with *Bacillus subtilis*, in the presence of rodent S9 mix. In this test AFB_1 exhibited a sharp dose dependency while STC was positive only in limited experimental conditions (i.e. lowest tested doses of 1–5 µg) (Ueno and Kubota, 1976).

Baertschi et al. (1989) analysed the mutagenicity of metabolically activated (by human CYP enzymes) STC and AFB_1 in the bacterial SOS-repair assay (using a *S. typhimurium* strain in which the *umuC* gene is linked to a lacZ reporter gene in plasmid pSK1002) in comparison with DNA adducts generated *in vitro* in isolated calf DNA. The level of guanyl-N7 adducts was in the order AFB_1 > STC (approximately 1.5 fold higher) while the order of the *umu* response was STC > AFB_1. Therefore, the SOS response per molecule of guanyl-N7 adduct was higher for STC than AFB_1. The authors concluded that the conformations of the structurally related AFB_1- and STC-induced guanyl-N7 adducts may prove to be significantly different in DNA thus accounting for the divergent biological responses. These findings were also confirmed by Krivobok et al. (1987), who tested STC with the SOS chromotest on *Escherichia coli*.

STC induced a dose-dependent increase in mutation frequency (resistance to L-canavanine) in yeast expressing rat CYP2B1 (Black et al., 1992).

Noda et al. (1981) showed induction of 8-azaguanine (8AG)-resistant mutations by STC in Chinese hamster ovary (CHO) cells which was greatly increased in the presence of liver microsomes from rats induced with phenobarbital. Reiners et al. (1983) showed that STC was weakly mutagenic at the hypoxanthine–guanine phosphoribosyltransferase (HPRT) locus in Chinese hamster lung V79 cells following activation by newborn mouse SENCAR keratinocytes used as a feeder layer.

Morita et al. (1991) showed that STC induces 6-thioguanine (6TG)-resistant mutations in the mouse mammary carcinoma FM3A cell line in the presence of rat S9 fraction. FM3A cells had also been used by Umeda et al. (1977), who compared the mutagenicity of various mycotoxins. The induction of 8AG-resistant mutations by STC was higher than that of AFB_1 but only at highly cytotoxic doses. STC as well as AFB_1 showed a weak induction of DNA single-strand breaks at high concentrations. In the same study STC induced high levels of chromosome aberrations.

DNA repair was measured in cultured DNA repair-deficient and normal fibroblasts derived from a xeroderma pigmentosum patient (XP-E) and a control person, respectively (Stich and Lashies, 1975). XP-E cells are defective in the nucleotide excision repair (NER), which plays a major role in the repair of bulky adducts. The cytotoxicity, chromosome breaking activity and DNA repair synthesis (measured as unscheduled DNA synthesis (UDS)) of STC, was greatly increased in the presence of liver S9 fractions isolated from different species in both normal and XP-E fibroblasts. Owing to the defect in NER, XP-E cells responded to the activated STC and AFB_1 with a lower level of UDS compared to normal cells, but higher levels of chromosomal damage and lethal effects.

The induction of UDS was measured in metabolically competent primary hepatocytes from mice and rats exposed to STC (and other mycotoxins) by Mori et al. (1984). STC was clearly positive, inducing a high UDS response. A variety of STC-related compounds were also positive in a DNA repair test in a primary hepatocyte culture from rats (Mori et al., 1986).

Crofton-Sleigh et al. (1993) showed that STC is positive in the micronucleus assay in the human lymphoblastoid $TK^{+/-}$ MCL-5 cell line that constitutively expresses a relatively high level of native CYP1A1, four other human cytochromes (CYP1A2, CYP2A6, CYP3A4 and CYP2E1) and microsomal epoxide hydrolase, carried as cDNAs in plasmids. Compared with AFB_1 the numbers of micronuclei were similar or higher for STC.

A potent induction of micronuclei was observed in V79 Chinese hamster cells genetically engineered to express rat CYP2B1, CYP1A1 or CYP1A2 (Ellard et al., 1991). STC induced predominantly kinetochore-negative micronuclei consistently with the induction of chromatid breaks (Ellard and Parry, 1993).

STC induces chromosome aberrations and sister chromatid exchanges (SCEs) in experimental animals *in vivo*. For example, STC induced increased SCE frequencies in bone marrow cells of female Swiss albino mice at all doses tested (0.06–6 mg/kg b.w.; i.p.) (Curry et al., 1984), and Ueda et al. (1984) found STC-induced chromosomal aberrations (chromatid breaks and gaps) in bone marrow cells in rats after the i.p. administration of 31.2 mg/kg b.w.

In the fish species *Oreochromis niloticus* (Nile tilapia), STC (1.6 µg/kg, twice a week for 4 weeks) induced a significant decrease of body weight and an increase in the frequency of micronucleated red blood cells as well as chromosomal aberrations in the kidney (Abdel-Wahhab et al., 2005).

In conclusion, STC is mutagenic in both bacterial and mammalian cells after metabolic activation. It induces chromosomal damage both *in vitro* upon metabolic activation and *in vivo* in experimental animals. Various studies aimed to compare the genotoxicity of STC and AFB_1. 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.

7.2.6. Chronic toxicity and carcinogenicity

The CONTAM Panel identified long-term carcinogenicity studies for STC in mice, rats, Mongolian gerbils, fish and monkeys.

7.2.6.1. Oral administration

Mouse

Two carcinogenicity studies on oral exposure in mice are available and details are summarised in Appendix D, Table D1.

Pulmonary tumours (adenomas and/or adenocarcinomas) were the main finding in ICR white Swiss mice of both sexes (18 males and 10 females in the treatment group) exposed to STC-containing feed

(Zwicker et al., 1974). Commercially obtained STC was added at a concentration of 5 mg STC/kg to feed, corresponding to approximately 0.75 mg STC/kg b.w. per day using the default factor of 0.15 as recommended by EFSA (2012). The intermitted treatment lasted for 54–58 weeks in total (two weeks of treatment followed by two weeks of uncontaminated diet). In mice fed STC, the first pulmonary neoplasms were seen at 50 weeks of exposure. Cumulative tumour incidence (until 58 weeks) reached 84 % (21/25) in the treatment group compared with 11 % (4/37) in the control group. The observed adenomas were mostly multiple, located deeply in the parenchyma without any obvious association with the airways. Adenocarcinomas occurred with much higher frequency in females (8/10) than in males (1/15). They were grossly described as large tan–white, firm raised masses which occasionally occupied the entire lobe. In the large tumours, central areas were necrotic, and these tumours invaded exclusively adjacent pulmonary tissue. There was a marked proliferation of large anaplastic epithelial cells with frequent mitotic figures.

Enomoto et al. (1982) exposed female BDF1 mice to STC-containing feed at a concentration of 30 or 120 mg STC/kg feed (unknown purity), corresponding to approximately 4.5 and 18 mg STC/kg b.w. per day using the default factor of 0.15 as recommended by EFSA (2012). However, the highest dose is an overestimation since the authors noted that the feed intake in this group was reduced. The control group consisted of 50 animals, and the low dose group of 55 animals which were exposed to a diet containing 30 mg STC/kg feed for 55 weeks. The third group of 55 animals were given a diet with 120 mg STC/kg feed for 40 weeks, then a control diet for 4 weeks (to compensate for the poor intake of the STC contaminated feed) and then again the diet with 120 mg STC/kg feed for another 11 weeks. Five animals of each group were killed at week 43, 10 animals at week 68 and the remaining survivors at week 73. The reported cumulative tumour incidence can be summarised as follows: in the control group, 2 animals developed either a leiomyoma or leiomyosarcoma of the uterus. No other tumours were observed. In the low dose group, angiosarcomas in the liver were observed in 34 out of 53 (64 %) animals and hepatic haemangioendotheliomas in 14 animals (26 %). In addition, angiosarcomas were observed in the brown fat (6 animals; 11 %), the ovary (1 animal) and the lung (1 animal). In the same dose group 10 animals also showed other tumours, such as lung adenoma (5), hepatocellular adenoma (1), HCC (1), papilloma of the oral cavity (1), leiomyosarcoma of the uterus (1) and leukaemia (1). In the high dose group, no angiosarcomas were observed in the liver, but four out of 55 animals (7 %) had developed hepatic haemangioendotheliomas. Angiosarcomas were observed in the brown fat (27) and in the ovaries (3). In this group 17 animals showed additional tumours, such as lung adenomas (12), hepatocellular adenomas (2), sarcoma of the uterus (1), leiomyoma of the uterus (1) and squamous cell carcinoma of the auditory gland duct (1). The authors described the tumours in the liver and the brown fat as typical vascular malignancies based on complementary investigation with electron microscopy. In summarising their results, the authors conclude that 72 % of the 104 STC-treated female mice surviving 43 weeks had one or more tumours. The first tumour appeared at week 43. No vascular tumours were observed in the control group and according to the authors this was the first report of vascular tumours in the brown fat in mice.

Rat

Five carcinogenicity studies on oral exposure in rats are available and details are summarised in Appendix D, Table D2.

Among a series of toxicity studies from which data were reported in Section 7.2.2, Purchase and van der Watt (1970a) performed also a carcinogenicity study. STC in this experiment was produced by *Bipolaris* spp. on maize meal, and the purity was about 88 %. Starting at weanling age, three groups of rats were exposed via the diet for six months to STC at concentrations of 10, 20 or 100 mg/kg feed, corresponding to 0.5, 1 and 5 mg STC/kg b.w. per day using the default factor of 0.05 recommended by EFSA (2012). The authors calculated a daily intake of 0.15, 0.3 and 1.5 mg/rat. Thereafter, dietary concentrations were increased to 15, 30 and 150 mg/kg feed, respectively for another six months (corresponding to 0.75, 1.5 and 7.5 mg STC/kg b.w. per day using the default factor of 0.05 recommended by EFSA (2012)). After these treatments, rats were placed on a normal diet until the end of the experiment at 123 weeks. Three other groups were given STC by gastric intubation at

doses of 0.15, 0.3 or 1.5 mg/rat (dissolved in 0.5 mL sunflower seed oil) five days a week for 52 weeks. One control group received sunflower oil five days a week for 52 weeks intragastrically, and another group received no treatment. Following dietary exposure to STC 0/9, 8/9, 10/10 and 3/3 animals showed HCC at the starting doses of 0, 0.15, 0.3 and 1.5 mg/rat, respectively, whereas the respective incidences were 0/10, 4/10, 5/9 and 9/9 following intragastric administration of STC. Histological investigations showed all features of a typical HCC with necrosis in the centre of the tumour mass. The amount of liver fibrosis varied considerably, but there was no evidence of a significant bile duct proliferation, as observed following exposure to AFB_1. In eight rats with primary HCC, other tumours were observed in liver, uterus, ovary, spleen and omentum. Acanthotic changes were present in the stomach of 85 % of the STC-treated animals.

In a study of Kempff et al. (1973) male Wistar rats were treated with STC (purity unknown) by gavage (20 mg/kg b.w.) once a week for 48 weeks. During the experimental period, four animals were sacrificed at approximately six week intervals (in total seven time points) to measure the activity of liver nuclear acid and alkaline DNase activity. After 48 weeks, nodular hyperplasia and foci were observed in the liver sections of these animals.

A two-year carcinogenicity study was performed by Ohtsubo et al. (1978) in male Donryu rats (six weeks old) fed *A. versicolor*-contaminated rice. The STC concentration in mouldy rice was determined using gas–liquid chromatography, but the purity of STC was not reported. Three groups each of 20 animals were given 0, 5 or 10 mg STC/kg feed, respectively for up to 101 weeks, corresponding to 0, 0.25 and 0.5 mg STC/kg b.w. per day using the default factor of 0.05 recommended by EFSA (2012). The authors calculated an equivalent exposure dose of 75 or 150 µg/rat, respectively for the two dose groups. The first animal with a tumour died after 66 weeks when in each of the two treatment groups the number of animals had declined from 20 to 13 animals and in the control group from 20 to 17 (effective group size) due to chronic bronchopulmonary infection (bronchitis, bronchopneumonia, bronchiectasis and pulmonary abscess). In the high dose group the survival rate dropped steeply between days 71 and 86 weeks of feeding, and in the low-dose group between 86 and 100 weeks. A high percentage of animals had hepatic tumours in the low-dose (11/13; 85 %) and in the high-dose group (12/13; 92 %). Histological investigations classified the tumours in most cases as HCCs and in three cases as haemangiosarcomas. Tumours were in almost all cases multiple and associated with hyperplastic nodules without peritoneal dissemination and cirrhosis. Tumours were large and pulmonary metastases were seen in three rats of each treatment group.

Terao et al. (1978) investigated STC carcinogenesis in male Wistar rats. Rats were given a diet containing 10 mg STC/kg feed (n = 15) for 54 weeks, followed by an observation period of another 15 weeks (total period 69 weeks). This concentration corresponds to a dose of 0.5 mg STC/kg b.w. per day using the default factor of 0.05 as recommended by EFSA (2012). The controls (n = 30) were given the basal diet only. Compared to the controls which did not develop any tumour, eight out of 15 (53 %) animals developed HCC which were first observed after 54 weeks. No metastases were found.

Maekawa et al. (1979) investigated hepatic changes in male ACI/N rats giving a diet containing 0.1, 1.0 and 10 mg STC/kg feed and administered throughout their life. These concentrations correspond to doses of 0.005, 0.05 and 0.5 mg STC/kg b.w. per day using the default factor of 0.05 recommended by EFSA (2012). At the beginning of the experiment the rats were 11 weeks old and were divided into three experimental groups (n = 36 per group), and a control group receiving the basal diet (n = 12). The animals were treated and observed for a maximum of 122 weeks. Mean survival time varied from 87 weeks (highest dose group) to 105 weeks in the control group. However, large variation in individual survival times was observed, and the animals suffered from multiple tumours. Tumours were observed in 7 out of 11 animals (64 %), 13 out of 27 (48 %), 6 out of 29 (21 %) and 10 out of 26 (38 %) in the 0, 0.005, 0.05 and 0.5 mg STC/kg b.w. per day dose groups, respectively. Therefore a relationship of tumour incidence to the rat strain rather than to the STC exposure cannot be excluded. With respect to changes in liver tissue, one case (4 %) of an HCC in the 0.5 mg STC/kg b.w. per day dose group and three (12 %) and one (3 %) haemangiosarcomas in the 0.5 and 0.05 mg STC/kg b.w.

per day dose group, respectively, were observed. In addition, central necrosis and hyperplastic foci, which both exhibited a clear dose-dependent relationship, were observed in the liver. Hyperplastic foci consisted of normal or larger vacuolated or eosinophilic cells, not demarcated from surrounding cells and without disrupting the liver architecture. Hyperplastic nodules appeared only in the liver of three animals of the highest dose group.

Other rodents

Recent literature, particularly from Asian countries, where STC seems to be a common food contaminant) focuses on the potential initiation or promotion of gastric cancers by STC. In particular, the co-exposure to *Helicobacter pylori* (one of the most common gastric infections) and STC was studied by Ma et al. (2003). STC was given with the diet at a concentration of 100 and 1000 mg/kg feed to Mongolian gerbils (n = 196, divided into 5 treatment groups) for a period of 27 months. In the presence of a *Helicobacter pylori*-infection, dietary STC exposure enhanced the development of intestinal metaplasia of the gastric mucosa (Ma et al., 2003). Following the same hypothesis that STC could evoke gastric lesions and potential gastric carcinomas, Kusunoki et al. (2011) investigated the effect of a long-term administration of STC in aged Mongolian gerbils. To this end, male gerbils with an age of 75 weeks (approximately 50 % of the expected lifespan of 3 years recorded for Mongolian gerbils) were included in the study and allocated to three groups, i.e. controls (n = 11); the group receiving 0.1 mg STC/L drinking water (n = 7) and the group receiving 1 mg STC/L drinking water (n = 13). STC (> 98 % purity) was administered over a period of 24 weeks at the specified concentration in drinking water. The authors reported an exposure rate of 0.007-0.015 mg/kg b.w. for the lower concentration group (0.1 mg STC/L drinking water) and 0.088 – 0.132 mg/kg b.w. per day for the higher concentration group (1 mg STC/L drinking water). Histopathological investigations at the end of the exposure period showed an active gastritis in all treated animals (both groups) whereas the incidence of gastritis in the control animals was low (2 out of 11 animals). Erosions in the stomach were found in 7 out of 7 and 12 out of 13 animals, respectively, in the STC exposed gerbils, but in only 1 out of 11 animals of the control group. The incidence of hyperplastic polyps reached 71.4 % in the lower dose group and 61 % in the higher dose group. Intestinal metaplasia were found in all animals (100 %) of the low dose group, but only in 2 out of 13 animals (15.4 %) of the higher dose group and in none of the animals (0 %) of the control group. Immuno-staining showed a high incidence of PCNA (proliferating cell nuclear antigen, a marker of cell proliferation), p53 (tumour suppressor gene, considered to be a marker for a transformation into a malignant tumour growth) and MDM2 (cellular inhibitor of p53) in the affected areas of the gastric mucosa, interpreted by the authors as an indication for precancerous lesions. It should be noted that most of the effects, both histopathological changes and the impairment of cell cycle markers (immune-histochemistry) were not dose-dependent (Kusunoki et al., 2011).

Non-human primates

In 1975, the National Cancer Institute started a long-term study on 30 adult non-human primates (species not specified) treated orally once a week with STC at 1.0 and 2.0 mg/kg b.w. (corresponding to 0.14 and 0.29 mg/kg b.w. per day) dissolved in DMSO (Thorgeirsson et al., 1994) within a project initiated in 1961 to study chemical carcinogenesis in non-human primates. The report of Thorgeirsson et al. (1994) is one of several interim reports of this study which was still ongoing in the mid 1990s but for which no final report could be identified in the literature except another summary report of Schoeffner and Thorgeirsson (2000) indicating that the study may have been terminated in 1997. No specific unexposed control group was assigned to this experiment. Until 1994, 10 treated monkeys developed one or more malignant tumours, five in each STC exposed group. Tumours were located mostly in liver (seven HCC, two cholangiocarcinomas, one cholangiosarcoma). One monkey developed a kidney tumour (renal cell carcinoma). Some monkeys died with other diagnoses such as myocarditis, sepsis, toxic hepatitis, cirrhosis, bronchopneumonia, acute renal tubular necrosis, and necrotising colitis. The laparoscopy showed extensive liver damage in animals that survived (and were still treated) by the time the report was published.

Fish

Carcinogenicity of STC was studied in rainbow trout (*Salmo gairdneri*) exposed as 14-day-old embryos for one hour to an aqueous suspension of 0.5 mg/L STC or 0.5 mg/L AFB_1 (Hendricks et al., 1980). The authors reported that this treatment did not cause significant mortality but produced HCC after 12 months of observation. At this time point the incidence of HCC in STC-treated trout was 13/100 and in AFB_1-treated trout 53/100. The tumour incidence in STC-treated trout was not sex-related, tumours were solitary and no tumour occurred in the control group.

The carcinogenicity of STC was also studied in medaka (*Orysiae latipes*) (Terao, 1983). Hepatic tumours occurred in 29 % (2/7) of male and 72 % (8/11) of female fish fed with STC (5 mg/kg)-contaminated feed for 24 weeks.

7.2.6.2. Non-oral administration

Carcinogenicity of STC has been demonstrated in rats and mice after i.p., s.c. and dermal administration (Dickens et al., 1966; Fujii et al., 1976; Purchase and van der Watt, 1973; Terao, 1978). Further details, including doses and tumour incidence are shown in Appendix D, Table D3.

7.2.6.3. Concluding comments

In conclusion, tumourigenicity of STC was observed after oral, intragastric, i.p., s.c. and/or dermal administration in the animal species tested (rat, mouse, Mongolian gerbil, monkey and fish). After oral exposure, premalignant and malignant lesions such as HCC, haemangiosarcomas in the liver, angiosarcomas in the brown fat, lung adenomas and incidental findings in other organs were reported. The CONTAM Panel concluded that STC is genotoxic and carcinogenic.

7.2.7. Combined effects of sterigmatocystin and aflatoxins

STC and AFs can be produced by the same fungal species and share the same biosynthetic pathway and therefore combined effects of these mycotoxins are frequently discussed in the literature.

Richard et al. (1978) investigated STC and AFB_1 (4.2 or 0.01 mg/day, respectively) given orally alone or in combination to growing female guinea pigs (n = 10 animals per group) for two weeks. Weight loss was statistically significantly higher in all treated groups compared to controls, higher for STC given alone or in combination with AFB_1 ($P < 0.01$) than for AFB_1 alone ($P < 0.05$), but additive overall. The dose of STC was intended not to be lethal, but two animals died. Complement titre values were statistically significantly lower in the combined group than in the groups exposed to STC and AFB_1 alone. Comparing the serum proteins of the combination with controls, the decrease in alpha-2-globulin and β-globulin was statistically significant, although albumin increased. However, groups given STC and AFB_1 alone did not differ significantly from the control except concerning alpha-2-globulin. Additive effects were reported for alpha-2-globulin and β-globulin. Although the authors declared a synergism between STC and AFB_1 in their combined effect on increasing blood serum albumin concentration (control: 2.3; STC: 2.4; AFB_1: 2.6; and combination: 2.9 g/dL), the CONTAM Panel noted that this effect may still be indistinguishable from an additive effect, since the authors did not report the outcome of a statistical evaluation. Increased severity of hepatic lesions was not found in the combination group. Overall, this study does not give convincing evidence for a synergistic action of STC with AFB_1 based on the high doses chosen and the sizes of the observed effects.

In investigating the interaction between STC and AFs in poultry, a combination of STC (350 µg/kg feed) and AFB_1 (100 µg/kg feed) in the diet was given to chicks from the first day after hatching for four weeks, followed by a four-week recovery period. Decreased body weight gain and a higher mortality rate was reported compared with control chicks kept on a mycotoxin-free diet, as was an increase in relative weights of various organs, particularly spleen (Abdelhamid and Dorra, 1993). However, since no single mycotoxin group was included, no comment on synergistic or additive effects can be made.

Based on the limited data, the CONTAM Panel concluded that the combined effect of STC and AFB_1 in food and feed seems to be at most additive.

7.3. Adverse effects in livestock, fish and companion animals

7.3.1. Ruminants

7.3.1.1. Cattle

Ingestion of feed accidentally contaminated with 7.75 mg STC/kg feed by dairy cattle resulted in bloody diarrhoea, loss of milk production and, in some animals, death. Those surviving were reported to resume milk production, and for the diarrhoea to stop, when switched to an alternative feed. No information on duration of exposure was reported for this study (Vesonder and Horn, 1985). In a small study with dairy cows (two animals in the exposure group and one in the control group), dietary exposure to approximately 5 to 10 mg STC per animal per day during 2 weeks exerted no-observable-adverse health effects (van Egmond et al., 1978).

7.3.1.2. Sheep

Nine female Austrian sheep (hybrid 'Kärntner Bergschafe') of 65 kg b.w. and aged between three and four years were used in a feeding trial, with 3 sheep for control and 6 for STC exposure (Böhm and Sayed, 1994). STC was used in two different purities; crude STC which was the total *A. versicolor* culture including the growth medium, and purified STC which was the extracted STC from the *A. versicolor* culture. All six animals in the trial group were fed the following increasing amounts of crude STC over a total of 100 weeks: 2 mg STC/kg feed (consisting of 1 kg hay and 200 g barley per day) for six weeks, 3 mg STC/kg feed for the next four weeks, 6 mg STC/kg feed for another four weeks and then 12 mg STC/kg feed for another four weeks. Finally, the trial group was divided such that three sheep received 16 mg crude STC/kg feed and three sheep received 16 mg purified STC/kg feed for eight weeks. Six weeks after the start of the treatment, and thereafter every two weeks until the end of the trial, blood samples were analysed for haematological (erythrocytes, leucocytes, haematocrit, haemoglobin) and biochemical (aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltransferase (γ-GT), glutamate dehydrogenase (GLDH), lactate dehydrogenase (LDH), urea, creatinine, calcium, magnesium, phosphor, total protein, albumin, glucose) parameters. In addition, biopsies of the liver were taken at the end of the STC exposure period. Finally, six months after the last exposure, all animals were killed and subjected to a *post-mortem* histopathological examination. No significant differences were observed between the three control and the six treated animals, even when given the highest dose tested (16 mg STC/kg feed, equivalent to 0.3 mg/kg b.w. per day based on rough calculations).

7.3.2. Pigs

Only one study with pigs could be identified (Kovalenko et al, 2011). This publication is apparently a summary of a larger trial and describes, in addition to the monitoring of feed materials, feeding experiments with two groups of two months old piglets. Animals were given a naturally contaminated diet identified in the monitoring programme containing approximately 30 µg/kg feed (feed intake was not recorded). Clinical signs observed were decreased feed intake, depression, incidental diarrhoea and alteration in blood biochemical parameters such as transaminase values (ALT and AST), γ-GT and alkaline phosphatase were observed as well as increased concentration of bilirubin and urea. *Post mortem* findings including severe alterations of the liver tissue with multiple, often large, necrotic spots.

7.3.3. Poultry

During an STC feeding study, newly-hatched male Warren chicks (treatment group: n = 15; control: n = 10) received normal feed for 3 days and on day 4, 5 and 6 feed contaminated with STC in concentrations of 1.6, 1.6 and 0.8 g/kg feed, respectively. This resulted in 60 % mortality within eight days of feeding. The surviving chicks from this group were fed a control diet without STC for an

additional seven weeks. After this period, grey spots on the liver surface, an increase in the kidney mass, elevated serum alkaline phosphatase and decreased triglycerides were observed. Another group of chickens (n = 20) received a diet with an increasing STC concentration (control diet first week, 20 mg STC/kg feed in the second week, 40 mg STC/kg feed in the third week and 50 mg STC/kg feed until seven weeks; equivalent to 40, 61 and 73 mg/kg b.w. per day respectively based on rough calculations). The chickens displayed after seven weeks, severe liver cirrhosis and fatty degeneration. In addition, cellular necrosis and intercellular inflammation were diagnosed, and b.w. gains and feed conversion rates were reduced in comparison with the control group. Serum biochemical parameters were altered, such as increased AST, γ-GT and creatine kinase, and reduced total protein, triglycerides, and cholesterol in serum. However, ALT, LDH, GLDH, alkaline phosphatase, urea, creatinine and uric acid were not affected. Symptoms of anaemia were recorded in haematological findings (Sayed, 1993).

Salam and Shanmugasundaram (1983) reported observations of adverse effects following a single oral dose of 50, 100, 250 or 500 µg STC/chick (n = 5 per dose group), but this study is poorly reported and as such it is not possible to draw any conclusions. Birds given 100 µg STC were reported to die on day two and histopathological examination showed fatty changes in the liver and in the distal tubules and glomerular endothelium of the kidneys, but these changes were not attributed to any dose level.

In four published papers, Sreemannarayana et al. (1986a,b, 1987, 1988) report experimental data of repeated dose experiments for chicks with STC by i.p. injection.

Five-day-old chicks were given 0.3 mg (36 birds) or 0.6 mg (44 birds) STC/kg b.w. in a single dose by i.p. injection. All dosed chicks had dull ruffled feathers after eight hours and greenish droppings were observed in the 0.6 mg/kg b.w. dose group after 12 hours; in this dose group decreased serum protein albumin and increased LDH and ALT were also noted (Sreemannarayana et al., 1986a).

A group of 40 ten-day-old chicks were given 4.0 mg STC/kg b.w. by i.p. injection and compared with 32 age-matched birds acting as controls receiving the carrier (olive oil) alone. Deaths occurred between 18 and 35 hours after dosing. Blood was collected from all chicks at 24 hours and from the 16 surviving dosed chicks at 36 hours. The dosed birds had increased AST, ALT and LDH, and decreased serum albumin and total protein. Histopathological examination showed changes described by the authors as 'mild to severe' in liver, pancreas, kidney and lymphoid tissue (Sreemannarayana et al.,1986b).

An experiment was reported in which chicks (n = 32 per dose group), were given either the carrier alone (olive oil) or 1.0 mg STC/kg b.w. by single i.p. injection. The chicks were killed at 12 or 24 hours. No histopathological effects were noted at 12 hours but changes in kidney were noted in those birds killed 24 hours post administration. Specifically, haemorrhage, foci of degeneration and necrosis of the liver were observed, and the kidneys showed tubular degeneration and necrosis (Sreemannarayana et al., 1987).

STC administered to male chicks of at least 100 g b.w. (n = 12) by i.p. injection at doses of 0.5 or 0.7 mg STC/animal on days 11, 13, 15, 17 and 19 of age resulted in markedly reduced growth and also affected organ weights (Sreemannarayana et al., 1988). An increase in the relative size of the crop, proventriculus, gizzard, large intestine, kidney and pancreas and a decrease in the bursa of Fabricius were reported. Peritonitis was observed in the chicks given the high dose. In treated animals, the activity of serum AST and the number of granulocytes in blood were elevated. STC treatment decreased the concentrations of total serum proteins, albumin and potassium and the total number of white blood cells. It also decreased the concentration of dry matter, DNA, RNA and protein in liver, affected glycogen concentration, but had no effect on lipid concentration. A reduction in white blood cell (WBC) counts of 21 and 29 % was observed for the 0.5 and 0.7 mg/animal dose groups respectively. In addition, there was a 40 and 56 % reduction in agranulocytes and a 46 and 66 % increase in granulocytes, respectively, when compared with control animals. STC caused necrosis at the periportal area of liver and liver haemorrhages. Hepatocytes were not clearly demarked and the

cytoplasm was eosinophilic with pyknosis and karyorrhexis. Degenerative changes were noticed in the epithelial cells of bile ducts. In the high dose group, kidneys showed haemorrhages that were subcapsular and interstitial. Proximal and distal tubules were degenerated, with pyknotic changes in tubular cells.

In an *in vitro* study of the inhibitory effect of various mycotoxins and moulds on the movement of tracheal cilia from 1 day-old chicks, STC (Jesenská and Bernát, 1994) and extracts of *A. versicolor* (Pieckova and Jesenska, 1997) were among the most toxic mycotoxins in this trial.

In conclusion, STC is hepatotoxic (necrosis, haemorrhage, cirrhosis and foci of degeneration of the liver) and nephrotoxic (tubular degeneration and necrosis) in poultry.

7.3.4. Fish

Abdelhamid (1988) fed STC to carp for three weeks at dose levels of 0, 10, 50, 250 1250 µg/kg feed and reported a decreased growth rate and a decreased muscular protein content at all doses. The paper does not, however, report the number of fish per group, or relevant quantitative details.

As discussed in Section 7.2.6, STC is carcinogenic to 14-day-old rainbow trout (*Salmo gairdneri*) embryos exposed for one hour to an aqueous suspension of 0.5 µg STC/L (Hendricks et al., 1980).

In Egyptian Nile tilapia (*Oreochromis niloticus*) fish, oral treatment with STC (1.6 µg/kg b.w., twice a week for four weeks, n = 8) caused after three days darker coloration, unbalanced swimming and the death of 25 % (2/8) of treated fish (Mahrous et al., 2006). In the gills of STC-treated animals, hyperplasia, lamellar oedema and haemorrhages were found. Haemorrhages were seen in branchial, hepatic and splenic tissues, and necrosis in hepatic and splenic tissue. In some cases, lesions included lysis of hepatocytes. STC caused also the appearance of eosinophilic granular cells and melanophores infiltrating the area of hepatopancreas. In this fish species the same dose statistically significantly increased chromosome aberrations (chromatid and chromosome breaks) in kidney tissues compared to controls (Abdel-Wahhab et al., 2005).

In conclusion, toxicity of STC has been demonstrated in several fish species. Pathological alterations included haemorrhages and oedema of the gills and haemorrhages and eosinophilic infiltrations in the hepatopancreas.

7.3.5. Other animal species

No data on adverse effects of STC could be identified for other farm livestock and companion animals.

7.4. Observations in humans

Various studies conducted particularly in China and other Asian countries, suggest a correlation between exposure to STC and the prevalence of gastric and liver cancer. For example, Lou et al. (1995) described that both the contamination rate and the content of STC in grains, were significantly higher in high incidence areas for gastric cancers than in a low incidence area in China. These findings have initiated a variety of further studies that are summarised in Sections 7.2.6.1 and 7.5. Comparable findings were reported in a study that measured the concentration of STC and STC adducts in biological material of patients with liver and stomach cancer diagnosed in the Chinese Academy of Medical Sciences Cancer Hospital in Beijing, China, and in healthy volunteers (Tian et al., 1995). The concentration of STC was measured in 28 samples of cancerous and pericancerous tissues of patients, 20 urine samples of patients and 10 of healthy persons and 14 blood samples of patients and 13 of healthy persons. Using indirect competitive ELISA with an LOD of 20 µg STC/kg, STC was found in the blood of 4 out of 13 patients (range 65–113 µg STC/kg) and in 1 (68 µg STC/kg) out of 14 healthy persons. In urine, STC concentrations were below the LOD in all samples tested. STC–DNA adducts were found in 14 out of 28 tissues of tumours collected from 12 patients. In a more recent paper, a study with 166 human patients, divided into 3 groups including controls (55 patients), patients with

liver cirrhosis (58 individuals) and patients with liver cancer (HCC) (53 individuals) is described (Hutanasu et al., 2011). In all patients, the concentration of STC in blood serum (measured by HPLC) was determined together with markers of liver function. STC was detected in 26.2 % of all samples, with a statistically significant ($p < 0.001$) higher prevalence in patients with liver cirrhosis and liver cancer. STC concentration varied between 0.01 and 0.005 ng/mL in blood in control subjects and reached values up to 2.02 ng/mL in blood and 9.39 ng/mL in urine in patients with HCC. In addition, a strong correlation between the presence of alfa-fetoprotein (a tumour marker) and STC was found in patients with liver cancer. The authors suggest that these findings may indicate a role of STC in the pathogenesis of liver cancer.

As tumour formation occurs at some latency after exposure to a substance that is genotoxic and carcinogenic and co-exposure to other carcinogens cannot be excluded, the above-mentioned data do not provide conclusive evidence that the described liver and stomach cancers have indeed been induced by dietary exposure to STC.

The CONTAM Panel noted that these sparse data provide evidence of recent exposure of STC in the relevant region and may support the use of STC concentration and DNA adducts in blood as biomarkers of recent exposure, but cannot inform on the risk of STC.

7.5. Modes of action

The first observation of covalent binding of STC to DNA was made following incubation of calf thymus DNA with STC in the presence of phenobarbital-induced rat liver microsomes (Essigmann et al., 1979). Spectral and chemical data identified the adduct as 1,2-dihydro-2-(N^7-guanyl)-1-hydroxysterigmatocystin, the guanine and hydroxyl moieties being in a *trans* configuration. The structure and stereochemistry of this adduct indicated that the exo-STC-1,2-oxide was the metabolite that reacted with DNA, and the quantitative yield of adducts indicated that this metabolite was a major product of the *in vitro* metabolism of STC. Formation of STC adducts was also detected when isolated rat livers were perfused with STC (Essigmann et al., 1980). A dose-dependent formation of DNA adducts of STC was found in the concentration range between 1 and 3 mg STC per liver. Male Fischer 344 rats were administered STC i.p. and sacrificed 2 hours post-dosing (Reddy et al., 1985). A dose-dependent STC–DNA adduct formation was detected in the rat liver over a 27-fold range of administered STC (0.33–9 mg/kg b.w. i.p.). In addition, STC–DNA adducts, formed in rats given 9 mg/kg b.w. i.p. were found to persist up to 105 days after treatment at a level of 0.5 % of the two-hour value. The disappearance of STC–DNA adducts showed a triphasic profile: a first, rapid phase during 24 hours ($t_{1/2}$ = 12 h), a second phase of slow decline from day 1 to 14 days ($t_{½}$ = 7 days) and extremely slow decline from day 14 to day 105 post treatment ($t_{½}$ = 109 days). In liver samples of male Fischer rats (three per group) treated with a single STC dose (1, 4, 8 and 16 mg/kg b.w., i.p.) and collected 24 hours after dosing, a linear dose–response relationship of STC–DNA adducts was found using ELISA and HPLC (Olson and Chu, 1993b).

Reactions of the epoxides of AFB_1 and STC with DNA were also analysed by Raney et al. (1992) by incubating calf thymus DNA with human liver microsomes. The rank order reported for the formation of N7-guanyl adducts by Baertschi et al. (1989) was confirmed. The yield of AFB_1-induced N7 alkyl adducts was higher (approximately 4 fold) than that induced by STC and a correlation was observed with the number of revertants measured in the *S. typhimurium* TA98 strain suggesting that the N7-guanyl adducts induced by STC and AFB_1 have similar mutagenic potencies. The same pattern was seen with the products of AFB_1 and STC oxidation by human liver microsomes (Raney et al., 1992). The yield of epoxide-GSH conjugates and other oxidation products followed the order AFB_1 > STC. In this study aflatoxin Q_1 (AFQ_1; detoxification product) was the most refractory substrate and presented the lowest activity in the *umu* genotoxicity assay. It is important to note that several lines of evidence indicate that human CYP3A4 catalyzes the oxidation of AFB_1 to both AFQ_1 and AFB_1-8,9-oxide. Nothwithstanding that the unusually higher ability of STC to induce the SOS response as compared to AFB_1 (Baertschi et al., 1989) might be due to DNA adduct-specific properties

(e.g. replication block), the detoxification of AFB_1 to AFQ_1, that is weakly genotoxic in the *umu* system on a molar basis (Raney et al., 1992), should be considered as an alternative explanation.

The cytotoxicity of STC was studied in various mammalian cell lines (Bünger et al., 2004) showing a high degree of heterogeneity in the susceptibility to the toxic effects of STC. The primary mechanism of cell death in cultured cells is necrosis (Purchase and van der Watt, 1970b; Ueno et al., 1995). Cell death might be related to an impairment of mitochondrial ATP synthesis (uncoupling of oxidative phosphorylation) as described by Kawai et al. (1984).

In primary kidney epithelial cells, STC (2 mg/L) completely blocked mitosis and caused nuclear changes in 100 % of cells after 24 h of exposure (Engelbrecht and Altenkirk, 1972). The incorporation of 3H-thymidine into DNA was inhibited as well as that of 3H-uridine in RNA. The inhibitory effect of STC on liver RNA synthesis was confirmed *in vivo* in Wistar rats (n = 21) injected i.p. with 50 mg STC/kg b.w. and sacrificed ½, 1, 2, 3, 4, 16 and 48 hours after treatment (Nel and Pretorius, 1970). This treatment inhibited the incorporation of orotic acid in RNA in liver, with the maximum inhibition (60 %) one hour after treatment and recovery to the control values within four hours after treatment.

In a more recent study, Xing et al. (2011) investigated the effects of STC on the cell cycle of human gastric epithelium cells (GES-1). They observed that STC arrested GES-1 cells at the G2 phase of the cell cycle and linked this observation to the individual cyclins and their regulation by MAPK and PI3K signalling pathways. These findings are in line with a previous study, demonstration that STC alleviated the normal p53-mediated cell cycle arrest that occurs for example as a consequence of DNA damage by induction of the p53 target gene MDM2 (Xie et al., 2000).

Cell damage and necrosis exerted by STC could also be related to the induction of cellular oxidative stress. Male Wistar albino rats (n = 6) were fed *A. versicolor*-contaminated feed which contained toxin concentrations resulting in dose levels equivalent to approximately 0.2, 0.3, and 0.4 mg STC/kg b.w. per day for 30 days, to study the effects of STC on liver cytochrome P450-dependent monooxygenases, as well as on the production of reactive oxygen species (ROS) and lipid peroxidation (Sivakumar et al., 2001). Since the two higher dose groups had shown more than 50 % mortality, the maximum tolerated dose of 0.2 mg/kg b.w. was used for further studies. This treatment significantly increased the content of both cytochrome P450, cytochrome b5 and their related reductases along with the production of ROS, while the levels of reduced glutathione, ascorbic acid and α-tocopherol, and the thiol status declined. The activity of catalase was reduced, whereas the activities of the enzymes superoxide dismutase and glutathione peroxidase were increased. Histopathological changes included parenchymal cell degeneration and necrosis (without evidence for cellular apoptosis), and Kupffer cells proliferation. The authors concluded that STC may activate biotransformation processes in liver microsomes and suggested that lipid peroxidation should be considered as a secondary mechanism of STC toxicity. The study did not provide information on oxidative DNA damage in STC-treated animals.

In conclusion, the genotoxicity of STC is likely due to the formation of DNA adducts that, if unrepaired, increase the likelihood of mutation fixation. Moreover, most *in vitro* studies with purified DNA indicate that the level of induced N7-guanyl adducts is higher after AFB_1 than STC exposure, supporting the view that AFB_1 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*. Many of the *in vitro* assays have been conducted with rather high STC concentrations. Hence, the observed effects are of qualitative nature and their relevance for the assessment of the potential adverse effects of (low dose) dietary exposure to STC remains limited.

7.6. Dose–response assessment

STC exhibits genotoxic effects *in vitro*, *in vivo* and *ex vivo* and carcinogenicity of STC has been demonstrated after oral, i.p., s.c. and/or dermal exposure in the animal species tested (Appendix D,

Tables D1, D2 and D3). The CONTAM Panel evaluated the dose–response effects of STC using data from available carcinogenicity bioassays using oral administration of STC as described in Section 7.2.6.1 for mice, rats and monkeys and summarised in Table 3.

The two identified carcinogenicity studies in mice were not suitable for dose–response analysis since dosing was discontinuous (Enomoto et al., 1982) or only one dose group was used (Zwicker et al., 1974). The CONTAM Panel also noted that data in an unspecified monkey species were inappropriate for dose–response assessment since the authors did not report detailed tumour incidence for the two dose groups (see also Table 3).

With the exception of the study by Maekawa et al. (1979), the available studies in rats were unsuitable for dose–response assessment. The studies either used discontinuous dosing (Purchase and van der Watt, 1970a (oral substudy)), exhibited a high portion of intercurrent mortality from non-neoplastic respiratory disease in both dose groups (Ohtsubo et al., 1978), or there was only one treatment group compared with a control group (Kempff et al., 1973; Terao et al., 1978). Furthermore, there was no clear dose-response relationship in the oral substudy by Purchase and van der Watt (1970a) and the study by Ohtsubo et al. (1978). A benchmark dose (BMD) analysis, conducted by the CONTAM Panel (Appendix E1) for the intragastric substudy by Purchase and van der Watt (1970a), showed a range of lower 95 % confidence limit for a benchmark response of 10 % extra risk ($BMDL_{10}$) values from different accepted models that substantially exceeded one order of magnitude and large $BMD_{10}/BMDL_{10}$ ratios for some models, indicating that the data are not informative enough for determining a reference point (EFSA, 2009, 2011b).

The study of Maekawa et al. (1979) on hepatic changes in rats reported a dose-dependent incidence of liver tumours (Appendix D, Table D2). However, the Panel noted that these data did not provide an appropriate basis for risk characterisation since the study combined tumours of different origin (HCC and haemangiosarcomas and one tumour was not specified). The incidence of HCC (only one tumour-bearing animal in the highest dose group) could not be reasonably analysed for dose-response assessment. The Panel concluded that it was appropriate to conduct a BMD analysis on the incidence of haemangiosarcomas (zero tumour-bearing animals in the control and low dose group, one tumour-bearing animal at the mid dose group and three tumour-bearing animals at the high dose group) which is a relevant endpoint. The lowest $BMDL_{10}$ value obtained from the fitted models was 0.16 mg/kg b.w. per day with a BMD_{10} of 0.36 mg/kg b.w. per day (Appendix E2). However, the Panel noted that the number of animals with haemangiosarcomas made up only 11 % (n = 4) of the total number of tumour-bearing animals (n = 36) and that the overall tumour incidence in the control group was 64 %. Therefore, this $BMD_{10}/BMDL_{10}$ pair is based on a limited tumourigenicity database. A dose-response relationship was also observed for necrosis and hyperplastic foci and/or hyperplastic areas; these were not subjected to dose-response analysis since it is not current practice to use such data in risk characterisation of substances that are genotoxic and carcinogenic.

In contrast to STC, dose-response assessment for AFB_1 is based on the incidence of HCC (EFSA, 2007). For AFB_1, a BMD_{10} of 0.00041 mg/kg b.w. per day (0.41 µg/kg b.w. per day) with a $BMDL_{10}$ of 0.00017 mg/kg b.w. per day (0.17 µg/kg b.w. per day) were calculated for male Fischer rats, which was the most sensitive species and strain. The Panel noted that haemangiosarcomas were also observed in the liver of animals exposed to AFB_1 in the study of Ward et al. (1975).

The CONTAM Panel compared the carcinogenic potency of STC and AFB_1 by comparing the BMD_{10} values. Whilst these BMD_{10} values are based on different tumour types, the Panel considered that they indicate that the carcinogenic potency of STC is approximately three orders of magnitude lower than that of AFB_1.

Table 3: Summary of studies on oral carcinogenicity of sterigmatocystin (details are provided in Appendix D, Tables D1 and D2).

Strain	Dose (mg/kg b.w. per day)	Duration of the observation (weeks)	Incidence of liver tumours (initial number of animals)	Incidence of angiosarcomas in brown fat (initial number of animals)	Incidence of lung neoplasia (initial number of animals)	Incidence of unspecified tumours (initial number of animals)	Reference
Mouse							
ICR Swiss male	0	54-58			2/16 (19)		Zwicker et al. (1974)
	0.75	54-58			12/15 (18)		
ICR Swiss female	0	54-58			2/21 (21)		Zwicker et al. (1974)
	0.75	54-58			9/10 (10)		
BDF1	0	43/68/73	0[(e)], 0[(b)] and 0[(a)]/50	0/50	0/50[(h)]		Enomoto et al. (1982)
	4.5	43/68/73	34[(e)], 14[(b)] and 1[(a)]/55	6/55	5/55[(h)]		
	18	43/68/73	0[(e)], 4[(b)] and 0[(a)]/55	27/55	12/55[(h)]		
Rat							
Wistar	0	123	0/9 (10)[(a)]				Purchase and van der Watt (1970a)
	0.5 + 0.75[(d)]	123	8/9 (10)[(a)]				
	1.0 + 1.5[(d)]	123	10/10 (10)[(a)]				
	5.0 + 7.5[(d)]	123	3/3 (10)[(a)]				
Wistar	0[(c)]	123	0/10 (10)[(a)]				Purchase and van der Watt (1970a)
	0.5[(c)]	123	4/10 (10)[(a)]				
	1.0[(c)]	123	5/9 (10)[(a)]				
	5.0[(c)]	123	9/9 (10)[(a)]				
Donryu	0	101	0 and 0[(f)]/17 (20)		0/17 (20)		Ohtsubo et al. (1978)
	0.25	101	11 and 1[(f)]/13 (20)		3/13 (20)		
	0.5	96	12 and 2[(f)] /13 (20)		3/13 (20)		

Table 3: Continued.

Strain	Dose (mg/kg b.w. per day)	Duration of the observation (weeks)	Incidence of liver tumours (initial number of animals)	Incidence of angiosarcomas in brown fat (initial number of animals)	Incidence of lung neoplasia (initial number of animals)	Incidence of unspecified tumours (initial number of animals)	Reference
Wistar	0	69	0/30[(a)]				Terao et al. (1978)
	0.5	69	8/15[(a)]				
ACI/N	0	121	0[(a)] and 0[(f)]/11 (12)				Maekawa et al. (1979)
	0.005	121	0[(a)] and 0[(f)]/27 (36)				
	0.05	122	0[(a)] and 1[(f)]/29 (36)				
	0.5	118	1[(a)] and 3[(f)]/26 (36)				
Monkey							
Species not specified	0					1.5-80 %	Thorgeirsson et al. (1994)
	0.14	12.3 years				5/15	
	0.28	11.5 years				5/15	
	0.14 or 0.28		7/30[(g)]				

b.w.: body weight.
(a): hepatocellular carcinomas only;
(b): hepatic haemangioendotheliomas;
(c): intragastric, 5 days per week;
(d): dose first six months + dose second six months;
(e): angiosarcomas;
(f): hepatic haemangiosarcomas;
(g): hepatocellular carcinomas only, not differentiated by dose group;
(h): adenomas only.

8. Risk characterisation

8.1. Human health risk characterisation

To characterise the risk of substances that are genotoxic and carcinogenic, the MOE approach is the most appropriate procedure (EFSA, 2005; Barlow et al., 2006) and no health-based guidance value (e.g., a tolerable daily intake (TDI)) would be calculated. The occurrence results reported to EFSA were all left-censored and no reliable human dietary exposure to STC could be calculated. The absence of exposure data for the European population precludes the application of the MOE approach and therefore, the CONTAM Panel could not characterise the risk of STC for human health.

8.2. Animal health risk characterisation

In the absence of exposure data for livestock, fish and companion animals in the EU and given the limited knowledge on the adverse effects of STC, the CONTAM Panel could not characterise the risks of STC for animal health.

9. Guidance for appropriate analytical performance to collect occurrence data in food and feed

Since currently due to absence of exposure data for the European population, a risk characterisation is not possible for STC, the CONTAM Panel recommends to collect more occurrence data for STC in food and feed across European countries.

The EFSA Scientific Committee has concluded that an MOE of 10 000 or more for substances that are genotoxic and carcinogenic is of low concern for public health (EFSA, 2005). For a $BMDL_{10}$ of 0.16 mg/kg b.w. per day, an MOE of 10 000 would be reached with an exposure of 0.016 µg/kg b.w. per day or less which would thus be of low concern for public health. Consumption of grains and grain-based products according to EFSA's comprehensive database ranges from 2.0 to 3.7 g/kg b.w. per day for average consuming adults. The STC concentration in grains and grain-based products resulting in an exposure of 0.016 µg/kg b.w. per day would range from 4.3 to 8 µg/kg grains and grain-based products.

For average consuming toddlers, the consumption of grains and grain-based products according to EFSA's comprehensive database ranges from 5.9 to 10.5 g/kg b.w. per day. Therefore, the STC concentration in grains and grain-based products resulting in an exposure of 0.016 µg/kg b.w. per day would range from 1.5 to 2.7 µg/kg grains and grain-based products.

Based on these considerations, the CONTAM Panel recommends that data on the occurrence of STC in food should be generated with methods with an LOQ less than 1.5 µg/kg. The available information on toxicity and exposure in farm and companion animals is insufficient to draw similar conclusions for feed.

10. Uncertainty analysis

The EFSA Panel on Contaminants in the Food Chain concluded that the magnitude of the uncertainties due to the lack of exposure data precludes a risk assessment for STC in food and feed.

The Panel therefore provided advice on the relative carcinogenic potency of STC and AFB_1, and on the concentration of STC in grains that might lead to a dietary exposure considered to be of low concern for public health. There is considerable uncertainty in the $BMD_{10}/BMDL_{10}$ of STC used as the basis for this advice, due to the limitations in the available carcinogenicity studies.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

Background

- Sterigmatocystin (STC) is a polyketide mycotoxin that is produced by more than 50 fungal species, including *Aspergillus flavus*, *A. parasiticus*, *A. versicolor* and *A. nidulans*, of which *A. versicolor* is the most common source.

- STC shares its biosynthetic pathway with aflatoxins. *A. nidulans* and *A. versicolor* are apparently unable to biotransform STC into O-methylsterigmatocystin, the direct precursor of aflatoxin B_1 (AFB_1) and G_1 (AFG_1). Consequently, substrates colonised by these fungi can contain high amounts of STC, while substrates invaded by *A. flavus* and *A. parasiticus* contain only low amounts of STC as most is converted into AFs.

- Grains and grain-based products can contain STC due to fungal infestation at the post-harvest stage.

Methods of sampling and analysis

- Analytical methods for STC in food and feed based on liquid chromatography-mass spectrometry reach the lowest limits of detection (LOD). However, the sensitivity varies between individual matrices and whether a single or multi-analyte method is applied.

- So far, no certified reference materials are available for STC. In addition, no proficiency tests for STC analysis in food or feed could be identified.

Occurrence and effect of processing

- The EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) investigated the occurrence of STC in food and feed in the literature.

    - The available information in the literature on the occurrence of STC in food and feed is scarce.

    - Most of the earlier studies using methods with rather high LODs/limits of quantification (LOQ) report a high percentage of left-censored data (often 100 %) for food and feed.

    - Two recent studies, using a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method with an LOD of 0.15 µg/kg and an LOQ of 0.3 µg/kg, reported STC positive samples of bread and of grain intended for use as animal feed. While these data indicate that these methods are sufficiently sensitive to quantify STC in food and feed, the data were insufficient to use for a reliable exposure assessment.

    - STC has been occasionally reported to occur in green coffee beans, spices, nuts and beer. Contamination of cheese occurs particularly at the surface, following fungal spoilage during ripening and storage.

- Following an EFSA call for data, analytical results for 581 samples, including 247 food and 334 feed samples, were submitted by three Member States. In food, analytical results on STC were reported to be all below the LOD or below the LOQ. In feed, only four quantified results were reported.

- Food processing (e.g. milling, bread and cheese making, roasting) can result in a decrease of the STC concentration; however the extent depends on the type of food and the processing conditions.

Exposure

- The CONTAM Panel concluded that a reliable exposure assessment was not feasible either for humans or for animals, due to the high percentage of left-censored data (100 % for food and 99 % for feed).

Hazard identification and characterisation

Toxicokinetics

- The scarce information available suggests that absorption of STC is limited following oral exposure. STC is metabolised in the liver and lung by various cytochrome P450 enzymes into different hydroxymetabolites and its reactive exo-epoxide that readily forms DNA adducts. Excretion of both conjugated parent STC and its hydroxylated metabolites occurs via bile and urine.

- There are insufficient data to assess the rate of carryover of STC from ruminants into milk when ruminants are exposed to contaminated feed, and no information is available about the transfer of STC and/or its metabolites into other animal products such as meat and eggs.

Toxicity

- The acute oral toxicity of STC is relatively low (range 120–166 mg/kg body weight (b.w.)). Liver and kidneys are the target organs of acute toxicity.

- STC is hepatotoxic in rat, mouse, monkey and guinea pig. Incidence of hepatocellular necrosis and haemorrhages increase with dose and duration of exposure.

- In the kidney, hyaline degeneration, tubular necrosis and haemorrhages were described in rats and/or vervet monkeys exposed to STC.

- Results from *in vivo* and *in vitro* studies suggest that STC may have immunomodulatory activity, but firm conclusions cannot be drawn.

- STC is mutagenic in both bacterial and mammalian cell assays after metabolic activation. It induces chromosomal damage both *in vitro*, upon metabolic activation, and *in vivo* in experimental animals.

- 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.

Adverse effects in livestock, fish and companion animals

- In sheep, no signs of toxicity were observed in a feeding trial at the highest dose tested (16 mg STC/kg feed, estimated as equivalent to 0.3 mg/kg b.w. per day).

- Limited data are available for other ruminants, but a case report describes haemorrhages and bloody diarrhoea in cattle following exposure to STC.

- STC is hepatotoxic in poultry and pigs, and nephrotoxic in poultry.
- Toxicity of STC has been demonstrated in several fish species. Pathological alterations included haemorrhages and oedema of the gills and haemorrhages and eosinophilic infiltrations in the hepatopancreas.
- No data on adverse effects of STC could be identified for other animal species.

Observations in humans

- There are indications of an association between STC exposure and prevalence of gastric cancers in Asia, but so far these data are inconclusive.

Mode of action

- STC, upon metabolic activation, forms N7-guanyl DNA adducts which are likely to be responsible for the mutagenic effects.
- STC induces cytotoxicity, inhibition of cell cycle and mitosis, as well as an increased formation of reactive oxygen species and lipid peroxidation *in vivo*.

Dose–response modelling

- Despite the evidence on genotoxicity and carcinogenicity, only a limited tumourigenicity database was available for dose–response assessment. From the data available on haemangiosarcomas in the liver of male rats, a benchmark dose for a response of 10 % extra risk (BMD_{10}) of 0.36 and a lower 95 % confidence limit for a benchmark response of 10 % extra risk ($BMDL_{10}$) of 0.16 mg STC/kg b.w. per day were calculated.
- A comparison of the BMD_{10} of STC for the occurrence of haemangiosarcomas and that of AFB_1 for the occurrence of HCC suggested that the carcinogenic potency of STC is approximately three orders of magnitude lower than that of AFB_1.

Human risk characterisation

- Due to the absence of exposure data for the European population, the margin of exposure (MOE) approach for substances that are genotoxic and carcinogenic could not be applied for STC and therefore, the CONTAM Panel could not characterise the risk of STC for human health.

Animal risk characterisation

- In the absence of exposure data for livestock, fish and companion animals, and given the limited knowledge on the adverse effects of STC, the CONTAM Panel could not characterise the risk of STC for animal health.

Guidance for appropriate analytical performance to collect occurrence data in food and feed

- Based on the $BMDL_{10}$ (0.16 mg STC/kg b.w. per day) and an MOE of 10 000, the STC concentration in grains and grain-based products leading to an exposure of low health concern (0.016 µg/kg b.w. per day) would range from 1.5 to 8 µg/kg.
- The available information on toxicity and exposure in farm and companion animals is insufficient to draw similar conclusions for feed.

RECOMMENDATIONS

- More occurrence data on STC in food and feed across European countries need to be collected to allow assessment of dietary exposure. For food, methods with an LOQ of less than 1.5 µg/kg should be applied. For feed, the available information is insufficient to make a recommendation.

- The development of suitable certified reference materials and/or proficiency tests to support analytical methodology should be encouraged.

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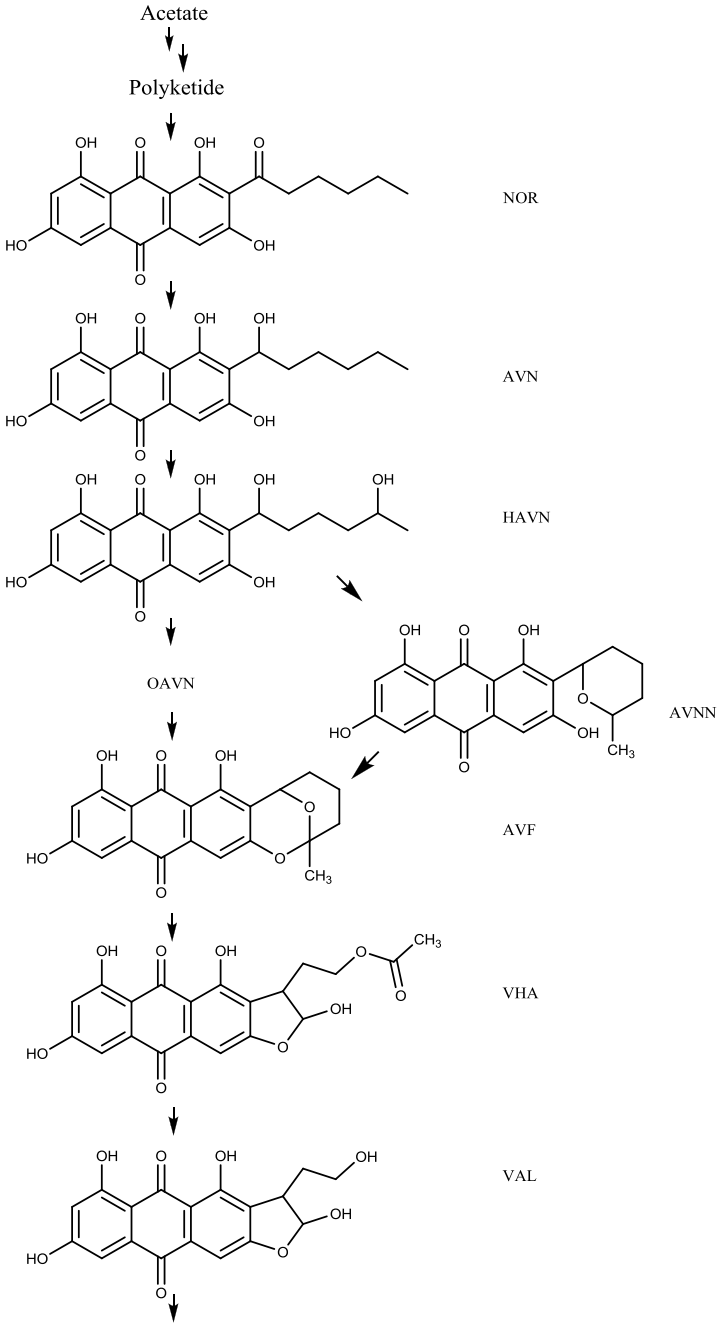
APPENDICES

Appendix A. Biosynthesis of aflatoxins and sterigmatocystin



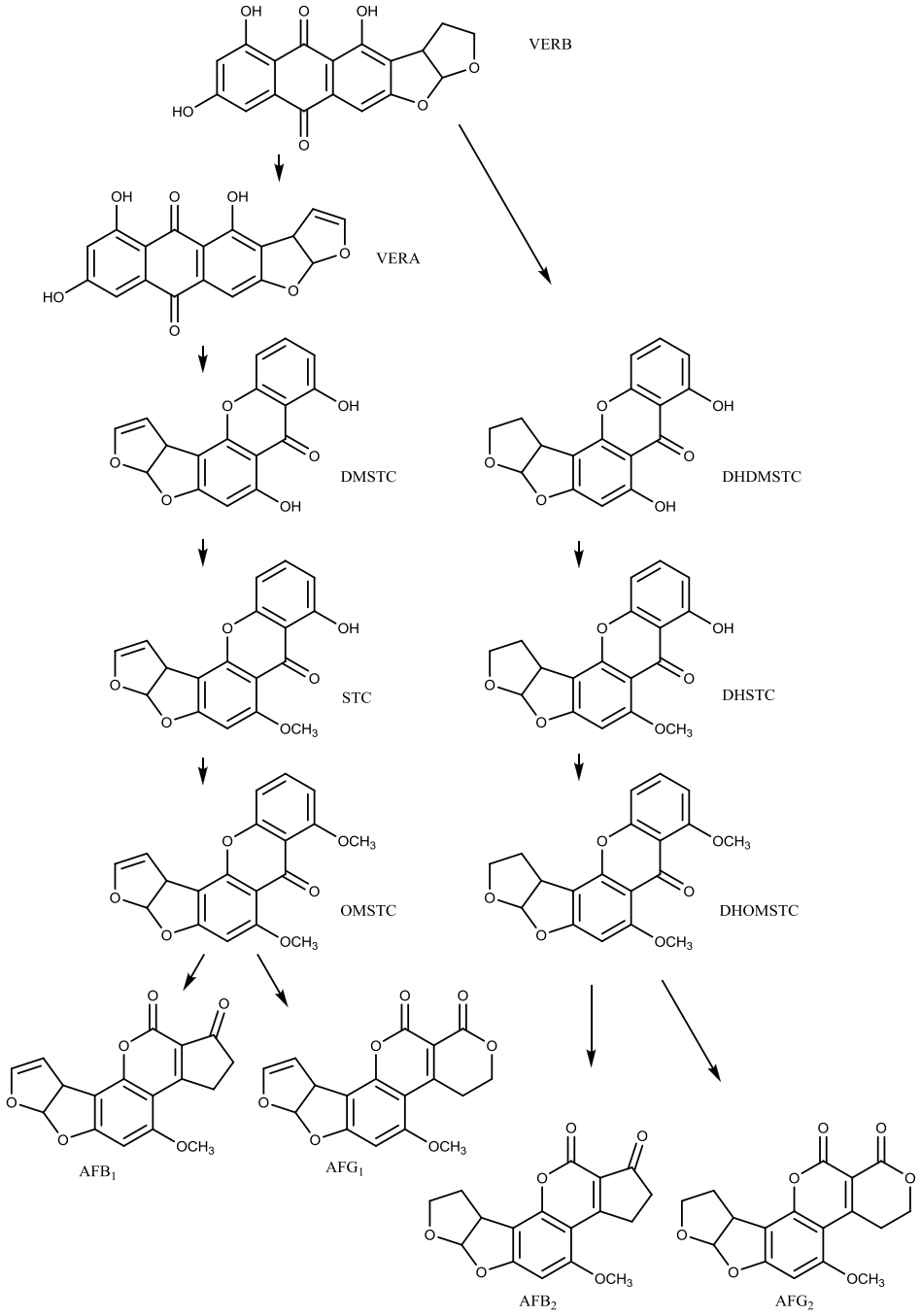


Figure A1: The aflatoxin and sterigmatocystin biosynthetic pathway (based on Yu et al., 2004).

Abbreviations: NOR: norsolorinic acid; AVN: averantin; HAVN: 5-hydroxyaverantin; OAVN: oxoaverantin; AVNN: averufanin; AVF: averufin; VHA: versiconal hemiacetal acetate; VAL: versiconal; VERB: versicolorin B; VERA: versicolorin A; DMSTC: demethylsterigmatocystin; DHDMSTC: dihydrodemethylsterigmatocystin; STC: sterigmatocystin; DHSTC: dihydrosterigmatocystin; OMSTC: O-methylsterigmatocystin; DHOMSTC: dihydro-O-methylsterigmatocystin; AFB_1: aflatoxin B_1; AFB_2: aflatoxin B_2; AFG_1: aflatoxin G_1; AFG_2: aflatoxin G_2.

Appendix B. Overview of previously reported literature data on occurrence of sterigmatocystin

Table B1: Overview of previously reported literature data on occurrence of sterigmatocystin in grains (indicating the final use (feed or food) if available).

Commodity	Country of sampling	Year[(a)]	*n*	Analytical method	LOD (µg/kg)	LOQ (µg/kg)	% LC	Descriptive statistics of numerical data (above LOD or LOQ) as reported by the authors (µg/kg)				Reference
								Min[(b)]	P50	Max	Mean	
Europe												
Maize (for food)	Germany	n.r.	30	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	Thurm et al. (1979)
Cereal grains (for food)	Poland	1975	12	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	Szebiotko et al. (1981)
Cereal grains (for food)	Poland	1976	37	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	Szebiotko et al. (1981)
Cereal grains (for food)	Poland	1977	62	TLC	n.r.	n.r.	98	n.r.	n.a.	n.a.	n.a.	Szebiotko et al. (1981)
Cereal grains (for food)	Poland	1978	296	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	Szebiotko et al. (1981)
Maize (for food)	UK	n.r.	29	n.r.	n.r.	n.r.	93	> 10	n.a.	n.r.	n.r.	Jarvis (1982)
Flake maize (for food)	UK	n.r.	2	n.r.	n.r.	n.r.	50	> 10	n.a.	n.a.	n.a.	Jarvis (1982)
Cereals (barley, wheat, oats) (for feed)	UK	1976–1979	523	TLC	20	n.r.	97	n.r.	n.r.	n.r.	n.r.	Buckle (1983)
Wheat (for feed)	Czechoslovakia	1980–1981	24	TLC	n.r.	n.r.	96	traces	n.r.	n.r.	n.r.	Bartos and Matyas (1983)
Barley (for feed)	Czechoslovakia	1980–1981	19	TLC	n.r.	n.r.	89	200	n.a.	400	n.r.	Bartos and Matyas (1983)
Maize (for feed)	Czechoslovakia	1980–1981	16	TLC	n.r.	n.r.	87	50	n.a.	n.r.	n.r.	Bartos and Matyas (1983)
Oats (for feed)	Czechoslovakia	1980–1981	10	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	Bartos and Matyas (1983)
Rye (for feed)	Czechoslovakia	1980–1981	5	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	Bartos and Matyas (1983)
Maize (for food)	UK	1987	20	n.r.	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	MAFF (1987)
Maize (for food)	Turkey	1986	50	TLC	20	n.r.	80	20	n.r.	n.r.	n.r.	Ozay and Heperkan (1989)
Maize (for food)	Bulgaria	1989–1990	n.r.	TLC	15	n.r.	100	n.a.	n.a.	n.a.	n.a.	Petkova-Bocharova et al. (1991)
Cereals (wheat, barley, oats) (for feed)	UK	n.r.	46	HPLC	n.r.	n.r.	83	n.r.	n.r.	n.r.	n.r.	Scudamore and Hetmanski (1995)
Barley (feed ingredient)	UK	1992	45	HPLC	15	n.r.	100	n.a.	n.a.	n.a.	n.a.	Scudamore et al. (1997)
Wheat (feed ingredient)	UK	1992	50	HPLC	15	n.r.	98	18	n.a.	n.a.	n.a.	Scudamore et al. (1997)
Barley (for food)	UK	1997–1998	20	HPLC	3	6	100	n.a.	n.a.	n.a.	n.a.	MAFF (1998)
Wheat (for food)	UK	1997–1998	20	HPLC	3	6	100	n.a.	n.a.	n.a.	n.a.	MAFF (1998)
Oats (for food)	UK	1997–1998	21	HPLC	3	6	100	n.a.	n.a.	n.a.	n.a.	MAFF (1998)
Rice (for food)	UK	1997–1998	21	HPLC	3	6	100	n.a.	n.a.	n.a.	n.a.	MAFF (1998)
Rye (for food)	UK	1997–1998	20	HPLC	3	6	100	n.a.	n.a.	n.a.	n.a.	MAFF (1998)
Maize (for food)	UK	1997–1998	20	HPLC	3	6	100	n.a.	n.a.	n.a.	n.a.	MAFF (1998)
Long grain rice (for food)	UK	2000	25	HPLC	3	6	100	n.a.	n.a.	n.a.	n.a.	FSA (2002a)
Easy cook rice (for food)	UK	2000	23	HPLC	3	6	100	n.a.	n.a.	n.a.	n.a.	FSA (2002a)
Basmati rice (for food)	UK	2000	18	HPLC	3	6	100	n.a.	n.a.	n.a.	n.a.	FSA (2002a)
Speciality rice (for food)	UK	2000	16	HPLC	3	6	100	n.a.	n.a.	n.a.	n.a.	FSA (2002a)
Brown rice (for food)	UK	2000	6	HPLC	3	6	100	n.a.	n.a.	n.a.	n.a.	FSA (2002a)

Table B1: Continued.

Commodity	Country of sampling	Year[a]	*n*	Analytical method	LOD (µg/kg)	LOQ (µg/kg)	% LC	Descriptive statistics of numerical data (above LOD or LOQ) as reported by the authors (µg/kg)				Reference
								Min[b]	P50	Max	Mean	
Europe (continued)												
Short grain rice (for food)	UK	2000	6	HPLC	3	6	100	n.a.	n.a.	n.a.	n.a.	FSA (2002a)
Flaked rice (for food)	UK	2000	3	HPLC	3	6	100	n.a.	n.a.	n.a.	n.a.	FSA (2002a)
Ground rice (for food)	UK	2000	3	HPLC	3	6	100	n.a.	n.a.	n.a.	n.a.	FSA (2002a)
Wheat (for food)	Latvia	2006	50	LC–MS/MS	0.15	0.30	82	0.7	n.r.	83	n.r.	Versilovskis et al. (2008a)
Barley (for food)	Latvia	2006	10	LC–MS/MS	0.15	0.30	80	0.7	n.a.	25	n.r.	Versilovskis et al. (2008a)
Oats (for food)	Latvia	2006	15	LC–MS/MS	0.15	0.30	100	n.a.	n.a.	n.a.	n.a.	Versilovskis et al. (2008a)
Buckwheat (for food)	Latvia	2006	10	LC–MS/MS	0.15	0.30	80	0.7	n.a.	25	n.r.	Versilovskis et al. (2008a)
Rye (for food)	Latvia	2006	10	LC–MS/MS	0.15	0.30	100	n.a.	n.a.	n.a.	n.a.	Versilovskis et al. (2008a)
Wheat (for food)	Latvia	2007	20	LC–MS/MS	0.15	0.30	60	1	n.r.	47	n.r.	Versilovskis et al. (2008a)
Barley (for food)	Latvia	2007	25	LC–MS/MS	0.15	0.30	56	1	n.r.	47	n.r.	Versilovskis et al. (2008a)
Oats (for food)	Latvia	2007	25	LC–MS/MS	0.15	0.30	76	1	n.r.	25	n.r.	Versilovskis et al. (2008a)
Buckwheat (for food)	Latvia	2007	25	LC–MS/MS	0.15	0.30	64	1	n.r.	47	n.r.	Versilovskis et al. (2008a)
Rye (for food)	Latvia	2007	25	LC–MS/MS	0.15	0.30	68	1	n.r.	25	n.r.	Versilovskis et al. (2008a)
Maize (for feed)	EU	2008	48	LC–MS/MS	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	Monbaliu et al. (2010)
Wheat (for feed)	EU	2008	30	LC–MS/MS	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	Monbaliu et al. (2010)
Grains	EU	2008-2011	367	LC–MS/MS	n.r.	n.r.	97	6.9	n.r.	574	n.r.	De Saeger, personal communication, 2012
North America												
Wheat (for feed)	Canada	1968	20	TLC	n.r.	n.r.	95	300	n.a.	n.a.	n.a.	Scott et al. (1972)
Barley (for feed)	Canada	1968	2	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	Scott et al. (1972)
Oats (for feed)	Canada	1968	3	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	Scott et al. (1972)
Rye (for feed)	Canada	1968	2	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	Scott et al. (1972)
Small grains	USA	n.r.	457	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	Stoloff (1976)
South America												
Maize	Brazil	1991–1992	130	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	Pozzi et al. (1995)
Polished rice	Brazil	1985–1986	52	TLC	15	35	100	n.a.	n.a.	n.a.	n.a.	Valente Soares and Rodriguez-Amaya (1989)
Parboiled rice	Brazil	1985–1986	8	TLC	15	35	100	n.a.	n.a.	n.a.	n.a.	Valente Soares and Rodriguez-Amaya (1989)
Asia												
Brown rice grains	Japan	n.r.	n.r.	TLC	n.r.	n.r.	n.r.	50	n.a.	450	n.r.	Sugimoto et al. (1977)
Brown rice grains	Japan	1975	n.r.	TLC	n.r.	n.r.	n.r.	3 800	n.r.	4 300	n.r.	Takahashi et al. (1984)
Brown rice grains	Japan	2005	48	HPLC-UV	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	Tanaka et al. (2007)

Table B1: Continued.

Commodity	Country of sampling	Year[(a)]	*n*	Analytical method	LOD (µg/kg)	LOQ (µg/kg)	% LC	Descriptive statistics of numerical data (above LOD or LOQ) as reported by the authors (µg/kg)				Reference
								Min[(b)]	P50	Max	Mean	
Asia (continued)												
Maize for feed	India	n.r.	50	n.r.	n.r.	n.r.	94	traces	n.r.	150	n.r.	Devi and Polasa (1982)
Wheat	India	1984–1986	30	n.r.	n.r.	n.r.	93	110	n.a.	145	n.r.	Pande et al. (1990)
Rice	India	1984–1986	30	n.r.	n.r.	n.r.	90	108	n.r.	157	n.r.	Pande et al. (1990)
Maize	India	1984–1986	22	n.r.	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	Pande et al. (1990)

UK: The United Kingdom; EU: European Union; USA: United States of America; n.r.: not reported; *n*: number of samples; TLC: thin-layer chromatography; HPLC: high-performance liquid chromatography; LC–MS/MS: liquid chromatography-tandem mass spectrometry; UV: ultraviolet; LOD: limit of detection; LOQ: limit of quantification; n.a.: not applicable; LC: left-censored data (values below the LOD or LOQ); Min: minimum; P50: 50th percentile; Max: maximum.

(a): Year of sample collection;

(b): When only one quantified sample was available, the concentration is reported as the minimum.

Table B2: Overview of previously reported literature data on occurrence of sterigmatocystin in food other than grains.

Commodity	Country of sampling	Year [(a)]	*n*	Analytical method	LOD (µg/kg)	LOQ (µg/kg)	% LC	Descriptive statistics of numerical data (above LOD or LOQ) as reported by the authors (µg/kg)				Reference
								Min[(b)]	P50	Max	Mean	
Grain-based products												
Europe												
Maize flour	UK	n.r.	13	n.r.	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	Jarvis (1982)
Maize-based breakfast cereals	UK	n.r.	6	n.r.	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	Jarvis (1982)
Oat-based breakfast cereals	UK	n.r.	6	n.r.	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	Jarvis (1982)
Wheat-based breakfast cereals	UK	n.r.	14	n.r.	n.r.	n.r.	93	7	n.a.	n.a.	n.a.	Jarvis (1982)
Breakfast cereals	UK	1997–1998	26	HPLC	3	n.r.	100	n.a.	n.a.	n.a.	n.a.	MAFF (1998)
Maize oils	UK	1997–1998	12	HPLC	3	n.r.	100	n.a.	n.a.	n.a.	n.a.	MAFF (1998)
Maize on the cob	UK	1997–1998	10	HPLC	3	n.r.	100	n.a.	n.a.	n.a.	n.a.	MAFF (1998)
Maize-based thickeners	UK	1997–1998	13	HPLC	3	n.r.	100	n.a.	n.a.	n.a.	n.a.	MAFF (1998)
Maize syrup	UK	1997–1998	5	HPLC	3	n.r.	100	n.a.	n.a.	n.a.	n.a.	MAFF (1998)
Polenta	UK	1997–1998	12	HPLC	3	n.r.	100	n.a.	n.a.	n.a.	n.a.	MAFF (1998)
Taco	UK	1997–1998	5	HPLC	3	n.r.	100	n.a.	n.a.	n.a.	n.a.	MAFF (1998)
Tinned sweet maize	UK	1997–1998	12	HPLC	3	n.r.	100	n.a.	n.a.	n.a.	n.a.	MAFF (1998)
Popcorn	UK	1997–1998	12	HPLC	3	n.r.	100	n.a.	n.a.	n.a.	n.a.	MAFF (1998)
Maize snacks	UK	1997–1998	21	HPLC	3	n.r.	100	n.a.	n.a.	n.a.	n.a.	MAFF (1998)
Cereal-based products	Italy	2002	85	HP-TLC	2 - 4	6 - 11	100	n.a.	n.a.	n.a.	n.a.	Stroka et al. (2004)
Rye bread	Latvia	n.r.	6	LC–MS/MS	0.15	0.30	83	2.4	n.a.	n.a.	n.a.	Versilovskis and Mikelsone (2008)
Rye-wheat bread	Latvia	n.r.	9	LC–MS/MS	0.15	0.30	89	7.1	n.a.	n.a.	n.a.	Versilovskis and Mikelsone (2008)
Wheat bread	Latvia	n.r.	14	LC–MS/MS	0.15	0.30	79	3.2	4.4	5.6	n.r.	Versilovskis and Mikelsone (2008)
South America												
Canned sweet maize	Brazil	1985–1986	32	TLC	15	35	100	n.a.	n.a.	n.a.	n.a.	Valente Soares and Rodriguez-Amaya (1989)
Maize flour	Brazil	1985–1986	27	TLC	15	35	100	n.a.	n.a.	n.a.	n.a.	Valente Soares and Rodriguez-Amaya (1989)
White meal maize	Brazil	1985–1986	6	TLC	15	35	100	n.a.	n.a.	n.a.	n.a.	Valente Soares and Rodriguez-Amaya (1989)
Yellow meal maize	Brazil	1985–1986	18	TLC	15	35	100	n.a.	n.a.	n.a.	n.a.	Valente Soares and Rodriguez-Amaya (1989)
Instant yellow meal maize	Brazil	1985–1986	13	TLC	15	35	100	n.a.	n.a.	n.a.	n.a.	Valente Soares and Rodriguez-Amaya (1989)
Popcorn	Brazil	1985–1986	15	TLC	15	35	100	n.a.	n.a.	n.a.	n.a.	Valente Soares and Rodriguez-Amaya (1989)

Table B2: Continued.

Commodity	Country of sampling	Year [a]	*n*	Analytical method	LOD (µg/kg)	LOQ (µg/kg)	% LC	Descriptive statistics of numerical data (above LOD or LOQ) as reported by the authors (µg/kg)				Reference
								Min[b]	P50	Max	Mean	
Grain-based products												
South America (continued)												
Yellow maize grits	Brazil	1985–1986	7	TLC	15	35	100	n.a.	n.a.	n.a.	n.a.	Valente Soares and Rodriguez-Amaya (1989)
Dried white maize	Brazil	1985–1986	12	TLC	15	35	100	n.a.	n.a.	n.a.	n.a.	Valente Soares and Rodriguez-Amaya (1989)
Cheese												
Europe												
Cheese	France	n.r.	235	TLC	n.r.	n.r.	99	45	125	330	n.r.	Lafont et al. (1979)
Cheese (Gouda and Edam)	The Netherlands	1977–1978	39	TLC	5	n.r.	77	5	n.r.	600	n.r.	Northolt et al. (1980)
Cheese	The Netherlands	n.r.	67	n.r.	n.r.	n.r.	73	Traces	n.r.	9 000	n.r.	Northolt and van Egmond (1982)
Cheese	Czechoslovakia	n.r.	66	TLC	n.r.	n.r.	95	7.5	n.a.	17.5	n.r.	Bartos and Matyas (1982)
Cheeses	Germany	n.r.	49	HPLC	20	n.r.	100	n.a.	n.a.	n.a.	n.a.	Nowotny et al. (1983)
Cheese	UK	1987	20	n.r.	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	MAFF (1987)
Cheese	UK	1997–1998	33	HPLC	3	n.r.	100	n.a.	n.a.	n.a.	n.a.	MAFF (1998)
Manchego cheese	Spain	n.r.	12	TLC	20	n.r.	100	n.a.	n.a.	n.a.	n.a.	Lopez-Diaz et al. (1996)
Cheese	Latvia	2008	8	LC–MS/MS	0.03	0.1	50	n.a.[c]	n.a.	n.a.	n.a.	Versilovskis et al. (2009)
Cheese	Belgium	2008	13	LC–MS/MS	0.03	0.1	77	0.52[d]	n.a.	1.23	n.r.	Versilovskis et al. (2009)
Africa												
Cheese	South Africa	n.r.	42	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	Luck et al. (1976)
Cheese	Egypt	n.r.	100	TLC	n.r.	n.r.	65	10	n.r.	63	24	Abd Alla et al. (1996); Metwally et al. (1997)
Nuts												
Europe												
Almonds	Spain	1986–1987	34	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	Jiménez et al. (1991)
Peanuts	Spain	1986–1987	38	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	Jiménez et al. (1991)
Hazelnuts	Spain	1986–1987	29	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	Jiménez et al. (1991)
Pistachio nuts	Spain	1986–1987	32	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	Jiménez et al. (1991)
Sunflower seeds	Spain	1986–1987	35	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	Jiménez et al. (1991)
Peanuts	UK	2000–2001	20	HPLC	n.r.	10	100	n.a.	n.a.	n.a.	n.a.	FSA (2002b)
North America												
Pecans	USA	1975	40	TLC	n.r.	n.r.	97	n.r.	n.a.	n.a.	n.a.	Schroeder and Hein (1977)
Africa												
Peanuts	Egypt	n.r.	40	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	El-Maghraby and El-Maraghy (1987)
Hazelnut	Egypt	n.r.	20	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	Abdel-Hafez and Saber (1993)

Table B2: Continued.

Commodity	Country of sampling	Year [a]	*n*	Analytical method	LOD (µg/kg)	LOQ (µg/kg)	% LC	Descriptive statistics of numerical data (above LOD or LOQ) as reported by the authors (µg/kg)				Reference
								Min[b]	P50	Max	Mean	
Nuts												
Africa (continued)												
Walnut	Egypt	n.r.	20	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	Abdel-Hafez and Saber (1993)
Untreated peanuts	Egypt	2004	20	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	Youssef et al. (2008)
Roasted peanuts	Egypt	2004	20	TLC	n.r.	n.r.	85	12.2	n.r.	16.8	n.r.	Youssef et al. (2008)
Roasted, salted peanuts	Egypt	2004	20	TLC	n.r.	n.r.	95	12.2	n.a.	n.a.	n.a.	Youssef et al. (2008)
Asia												
Almond	Saudi Arabia	n.r.	5	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	Abdel-Gawad and Zohri (1993)
Cashew nut	Saudi Arabia	n.r.	5	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	Abdel-Gawad and Zohri (1993)
Chestnut	Saudi Arabia	n.r.	5	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	Abdel-Gawad and Zohri (1993)
Hazelnut	Saudi Arabia	n.r.	5	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	Abdel-Gawad and Zohri (1993)
Pistachio nut	Saudi Arabia	n.r.	5	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	Abdel-Gawad and Zohri (1993)
Walnut	Saudi Arabia	n.r.	5	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	Abdel-Gawad and Zohri (1993)
Other products												
Europe												
Green coffee beans	Italy	n.r.	502	n.r.	n.r.	n.r.	99.8	1200	n.a.	n.a.	n.a.	De Palo et al. (1977)
Grapes and plums	Germany	n.r.	10	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	Thurm et al. (1979)
Apples and pears	Germany	n.r.	10	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	Thurm et al. (1979)
Bananas and oranges	Germany	n.r.	5	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	Thurm et al. (1979)
Sterilized fruits	Germany	n.r.	10	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	Thurm et al. (1979)
Fruit juices	Germany	n.r.	52	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	Thurm et al. (1979)
Apple juices	Germany	n.r.	12	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	Thurm et al. (1979)
Blackcurrant juice	Germany	n.r.	5	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	Thurm et al. (1979)
Cherry juice	Germany	n.r.	5	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	Thurm et al. (1979)
Green vegetables	Germany	n.r.	10	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	Thurm et al. (1979)
Tomatoes	Germany	n.r.	10	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	Thurm et al. (1979)
Sterilized canned vegetables	Germany	n.r.	5	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	Thurm et al. (1979)
Dark beer	Latvia	n.r.	9	HPLC	0.26 µg/L	0.68 µg/L	89	7.8 µg/L	n.a.	n.a.	n.a.	Versilovskis et al. (2008b)
Light beer	Latvia	n.r.	17	HPLC	0.26 µg/L	0.68 µg/L	94	4.0 µg/L	n.a.	n.a.	n.a.	Versilovskis et al. (2008b)
Asia												
Green coffee beans	Saudi Arabia	n.r.	30	TLC	n.r.	n.r.	93	11	n.a.	13	n.r.	Bokhari and Aly (2009)
Fennel	India	1984–1986	9	TLC	n.r.	n.r.	89	142	n.a.	n.a.	n.a.	Saxena and Mehrotra (1989)
Black Pepper	India	1984–1986	8	TLC	n.r.	n.r.	75	105	n.a.	125	n.r.	Saxena and Mehrotra (1989)

Table B2: Overview of previously reported literature data on occurrence of sterigmatocystin in food other than grains.

Commodity	Country of sampling	Year [a]	*n*	Analytical method	LOD (µg/kg)	LOQ (µg/kg)	% LC	Descriptive statistics of numerical data (above LOD or LOQ) as reported by the authors (µg/kg)				Reference
								Min[b]	P50	Max	Mean	
Other products												
Asia (continued)												
Turmeric	India	1984–1986	9	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	Saxena and Mehrotra (1989)
Coriander	India	1984–1986	9	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	Saxena and Mehrotra (1989)
Cinnamon	India	1984–1986	6	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	Saxena and Mehrotra (1989)
Cardamom	India	1984–1986	6	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	Saxena and Mehrotra (1989)
Greater cardamom	India	1984–1986	6	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	Saxena and Mehrotra (1989)
Indian cassia	India	1984–1986	6	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	Saxena and Mehrotra (1989)
Ammi	India	1984–1986	7	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	Saxena and Mehrotra (1989)
Cumin	India	1984–1986	8	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	Saxena and Mehrotra (1989)
Chilli	India	1984–1986	9	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	Saxena and Mehrotra (1989)
Yellow mustard	India	1984–1986	6	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	Saxena and Mehrotra (1989)
Indian mustard	India	1984–1986	7	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	Saxena and Mehrotra (1989)
Garlic	India	1984–1986	6	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	Saxena and Mehrotra (1989)
Clove	India	1984–1986	6	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	Saxena and Mehrotra (1989)
Africa												
Green coffee beans	South Africa	n.r.	2	TLC	n.r.	n.r.	50	1 143	n.a.	n.a.	n.a.	Purchase and Pretorius (1973)
Anise	Egypt	n.r.	5	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	El-Kady et al. (1995)
Black cumin	Egypt	n.r.	5	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	El-Kady et al. (1995)
Black pepper	Egypt	n.r.	5	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	El-Kady et al. (1995)
Caraway	Egypt	n.r.	5	TLC	n.r.	n.r.	40	14	n.r.	18	n.r.	El-Kady et al. (1995)
Cardamom	Egypt	n.r.	5	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	El-Kady et al. (1995)
Chinese cassia	Egypt	n.r.	5	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	El-Kady et al. (1995)
Cinnamon	Egypt	n.r.	5	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	El-Kady et al. (1995)
Clove	Egypt	n.r.	5	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	El-Kady et al. (1995)
Coriander	Egypt	n.r.	5	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	El-Kady et al. (1995)
Cubeb pepper	Egypt	n.r.	5	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	El-Kady et al. (1995)
Cumin	Egypt	n.r.	5	TLC	n.r.	n.r.	40	n.r.	n.r.	n.r.	11	El-Kady et al. (1995)
Eucalyptus	Egypt	n.r.	5	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	El-Kady et al. (1995)
Fennel	Egypt	n.r.	5	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	El-Kady et al. (1995)
Ginger	Egypt	n.r.	5	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	El-Kady et al. (1995)
Laurel	Egypt	n.r.	5	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	El-Kady et al. (1995)
Mastic	Egypt	n.r.	5	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	El-Kady et al. (1995)
Marjoram	Egypt	n.r.	5	TLC	n.r.	n.r.	80	n.r.	n.a.	n.a.	n.a.	El-Kady et al. (1995)
Nutmeg	Egypt	n.r.	5	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	El-Kady et al. (1995)
Peppermint	Egypt	n.r.	5	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	El-Kady et al. (1995)
Red pepper	Egypt	n.r.	5	TLC	n.r.	n.r.	40	10	n.r.	23	n.r.	El-Kady et al. (1995)
Rosemary	Egypt	n.r.	5	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	El-Kady et al. (1995)

Table B2: Continued.

Commodity	Country of sampling	Year [(a)]	*n*	Analytical method	LOD (µg/kg)	LOQ (µg/kg)	% LC	Descriptive statistics of numerical data (above LOD or LOQ) as reported by the authors (µg/kg)				Reference
								Min[(b)]	P50	Max	Mean	
Other products												
Africa (continued)												
Safflower	Egypt	n.r.	5	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	El-Kady et al. (1995)
Thyme	Egypt	n.r.	5	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	El-Kady et al. (1995)
White pepper	Egypt	n.r.	5	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	El-Kady et al. (1995)
North America												
Coffee beans	USA	n.r.	15	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	Stoloff (1976)
South America												
Raw cassava flour	Brazil	1985–1986	33	TLC	15	35	100	n.a.	n.a.	n.a.	n.a.	Valente Soares and Rodriguez-Amaya (1989)
Roasted cassava flour	Brazil	1985–1986	9	TLC	15	35	100	n.a.	n.a.	n.a.	n.a.	Valente Soares and Rodriguez-Amaya (1989)
Seasoned and roasted cassava flour	Brazil	1985–1986	3	TLC	15	35	100	n.a.	n.a.	n.a.	n.a.	Valente Soares and Rodriguez-Amaya (1989)
Dried beans	Brazil	1985–1986	61	TLC	15	35	100	n.a.	n.a.	n.a.	n.a.	Valente Soares and Rodriguez-Amaya (1989)

UK: The United Kingdom; EU: European Union; USA: United States of America; n.r.: not reported; *n*: number of samples; HPLC: high-performance liquid chromatography; TLC: thin-layer chromatography; HP-TLC: high performance thin-layer chromatography; LC–MS/MS: liquid chromatography–tandem mass spectrometry; LOD: limit of detection; LOQ: limit of quantification; LC: left-censored data (values below the LOD or LOQ); n.a.: not applicable; Min: minimum, P50: 50th percentile; Max: maximum.

(a): Year of sample collection.

(b): When only one quantified sample was available, the concentration is reported as the minimum.

(c): STC was detected in four samples above the LOD, but below the LOQ.

(d): Lowest quantified concentration. In one sample STC was detected but not quantified.

Table B3: Overview of previously reported literature data on occurrence of sterigmatocystin in feed other than grains.

Commodity	Country of sampling	Year [(a)]	n	Analytical method	LOD (µg/kg)	LOQ (µg/kg)	% LC	Descriptive statistics of numerical data (above LOD or LOQ) as reported by the authors (µg/kg)				Reference
								Min[(b)]	P50	Max	Mean	
Europe												
Feedstuffs	UK	1976–1979	811	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	Buckle (1983)
Hay	UK	1976–1979	157	TLC	n.r.	n.r.	99	< 40	n.a.	n.a.	n.a.	Buckle (1983)
Grass silage	UK	1976–1979	451	TLC	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	Buckle (1983)
Rice bran (feed ingredient)	UK	1992	40	HPLC	20	n.r.	100	n.a.	n.a.	n.a.	n.a.	Scudamore et al. (1997)
Maize gluten and products (feed ingredient)	UK	1992	50	HPLC	40	n.r.	100	n.a.	n.a.	n.a.	n.a.	Scudamore et al. (1997)
Cottonseed meal (feed ingredient)	UK	1992	21	HPLC	40	n.r.	100	n.a.	n.a.	n.a.	n.a.	Scudamore et al. (1997)
Rapeseed (feed ingredient)	UK	1992	25	HPLC	25	n.r.	100	n.a.	n.a.	n.a.	n.a.	Scudamore et al. (1997)
Sunflower meal (feed ingredient)	UK	1992	20	HPLC	20	n.r.	100	n.a.	n.a.	n.a.	n.a.	Scudamore et al. (1997)
Olive pulp (feed ingredient)	UK	1992	5	HPLC	25	n.r.	100	n.a.	n.a.	n.a.	n.a.	Scudamore et al. (1997)
Palm products (feed ingredient)	UK	1992	15	HPLC	40	n.r.	100	n.a.	n.a.	n.a.	n.a.	Scudamore et al. (1997)
Soya (feed ingredient)	UK	1992	20	HPLC	20	n.r.	100	n.a.	n.a.	n.a.	n.a.	Scudamore et al. (1997)
Peas/beans (feed ingredient)	UK	1992	15	HPLC	20	n.r.	100	n.a.	n.a.	n.a.	n.a.	Scudamore et al. (1997)
Manioc (feed ingredient)	UK	1992	10	HPLC	25	n.r.	100	n.a.	n.a.	n.a.	n.a.	Scudamore et al. (1997)
Rice bran (feed ingredient)	UK	n.r.	40	HPLC	n.r.	50	100	n.a.	n.a.	n.a.	n.a.	Scudamore et al. (1998b)
Maize gluten (feed ingredient)	UK	n.r.	40	HPLC	100	n.r.	100	n.a.	n.a.	n.a.	n.a.	Scudamore et al. (1998a)
Maize products (feed ingredient)(maize germs/brans, baby maize, maize meals, flaked maize, maize screens)	UK	n.r.	27	HPLC	20	n.r.	100	n.a.	n.a.	n.a.	n.a.	Scudamore et al. (1998a)
Maize silages	The Netherlands	2002–2004	140	LC–MS/MS	n.r.	50	100	n.a.	n.a.	n.a.	n.a.	Driehuis et al. (2008b)
Grass silages	The Netherlands	2002–2004	120	LC–MS/MS	n.r.	50	100	n.a.	n.a.	n.a.	n.a.	Driehuis et al. (2008b)
Wheat silages	The Netherlands	2002–2004	30	LC–MS/MS	n.r.	50	100	n.a.	n.a.	n.a.	n.a.	Driehuis et al. (2008b)
Maize and grass silages	The Netherlands	2005	47	LC–MS/MS	n.r.	50	100	n.a.	n.a.	n.a.	n.a.	Driehuis et al. (2008a)
compound feeds	The Netherlands	2005	72	LC–MS/MS	n.r.	50	100	n.a.	n.a.	n.a.	n.a.	Driehuis et al. (2008a)
ensiled by-products	The Netherlands	2005	29	LC–MS/MS	n.r.	50	100	n.a.	n.a.	n.a.	n.a.	Driehuis et al. (2008a)
feed commodities	The Netherlands	2005	8	LC–MS/MS	n.r.	50	100	n.a.	n.a.	n.a.	n.a.	Driehuis et al. (2008a)
forage products	The Netherlands	2005	13	LC–MS/MS	n.r.	50	100	n.a.	n.a.	n.a.	n.a.	Driehuis et al. (2008a)
Sow feed	EU	2008	4	LC–MS/MS	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	Monbaliu et al. (2010)
Feed unspecified	EU	2008-2011	282	LC–MS/MS	n.r.	n.r.	97	8.6	n.r.	17.2	n.r.	Monbaliu et al. (2010)
America												
Dairy cattle feed (maize, cottonseed, protein mix)	USA	1983	1	TLC	n.r.	n.r.	0	7750	n.a.	n.a.	n.a.	Vesonder and Horn (1985)
Africa												
Silages	Egypt	n.r.	40	TLC	n.r.	n.r.	95	n.r.	n.r.	n.r.	n.r.	El-Shanawany et al. (2005)

UK: The United Kingdom; EU: European Union; USA: United States of America; TLC: thin-layer chromatography; HPLC: high-performance liquid chromatography; LC–MS/MS: liquid chromatography–tandem mass spectrometry; LOD: Limit of detection; n.r.: not reported; LOQ: limit of quantification; LC: left-censored data (values below the LOD or LOQ); Min: minimum; n.a.: not applicable, P50: 50th percentile; Max: maximum.

(a): Year of sample collection.

(b): When only one quantified sample was available, the concentration is reported as the minimum.

Appendix C. Occurrence of sterigmatocystin in food and feed: call for data

Table C1: Distribution of 247 food samples across food groups[(a)].

Food group	*n*	LC	Mean UB[(b)] (µg/kg)
Grains and grain products			
Grains for human consumption	99	100 %	5.9
Grain milling products	16	100 %	3.6
Bread and rolls	119	100 %	5
Breakfast cereals	5	100 %	3
Fine bakery wares	2	100 %	3
Pasta (raw)	2	100 %	3
Snack food	4	100 %	3

n: number of samples; LC: proportion of left-censored results; UB: upper bound.
(a): It cannot be excluded that samples described in Section 4.1 (Previously reported literature data on the occurrence of STC) were also submitted to EFSA.
(b): Lower bound means were equal to zero.

Table C2: Distribution of 334 feed samples across feed groups[(a)].

Feed group	*n*	LC	Mean LB-UB[(b)] (µg/kg)	Max[(c)]
Cereal grains, their products and by-products				
Barley	39	100 %	5	
Maize	33	100 %	5	
Oats	2	100 %	5	
Triticale	4	100 %	5	
Wheat	45	100 %	5	
Forages and roughage, and products derived thereof				
Forage meal (grass meal, green meal)	2	100 %	5	
Forages and roughage, and products derived thereof (unspecified)	127	98 %	0.1–5.1	8.9
Lucerne (alfalfa)	1	0 %	28	28
Legume seeds and products derived thereof				
Peas	1	100 %	5	
Sweet lupins	1	100 %	5	
Miscellaneous				
Starch	1	100 %	5	
Oil seeds, oil fruits, and products derived thereof				
Cocoa husks	2	50 %	7–9.5	14
Linseed	2	100 %	5	
Palm kernel expeller	9	100 %	5	
Rape seed	5	100 %	5	
Sunflower seed	5	100 %	5	
Toasted soya beans	24	100 %	5	
Citrus pulp	9	100 %	5	
Other seeds and fruits, and products derived thereof	1	100 %	5	
Tubers, roots, and products derived thereof				
Potatoes	1	100 %	5	
Sugar beet	7	100 %	5	
Other feed	13	100 %	5	

n: number of samples; LC: proportion of left-censored results; LB: lower bound; UB: upper bound.

(a): It cannot be excluded that samples described in Section 4.1 (Previously reported literature data on the occurrence of STC) were also submitted to EFSA.

(b): .When only one value is shown, lower bound is zero and only upper bound is shown.

(c): Maximum values given only for feed groups with quantified results .

Appendix D. Carcinogenicity of sterigmatocystin in rodents

Table D1: Carcinogenicity of sterigmatocystin in mice after oral exposure (feeding).

Number of animals at beginning	STC concentration in feed (mg/kg feed)	Daily STC dose (mg/kg b.w.)	Length of treatment (observation) in weeks	Number of animals at end of experiment	First appearance of tumours at week	Organ	Number of animals with tumours	Type of tumour/ multiplicity if applicable[(b)]	Reference
ICR Swiss white, 3 weeks old, 11-16 g b.w. at start									Zwicker et al. (1974)
Male									
19	0	0	n.a.	16	n.r.	Lung	2	2 pulmonary neoplasm	
18	5	0.75[(a)]	54-58 wks, 2 wks on + 2 wks off	15	50	Lung	12	12 pulmonary neoplasm (incl. 1 adenocarcinoma)	
Female									
21	0	0	n.a.	21	n.r.	Lung	2	2 pulmonary neoplasm	
10	5	0.75[(a)]	54-58 wks, 2 wks on + 2 wks off	10	n.r.	Lung	9	9 pulmonary neoplasm (incl. 8 adenocarcinoma)	
BDF1mice, 5 weeks old, 0.016 kg b.w. at start									Enomoto et al. (1982)
Female									
50	0	0	n.a. (43, 68 or 73 wks)	34	73	Uterus	2	1 leiomyosarcoma 1 leiomyoma	
55	30	4.5[(a)]	55 wks (43, 68 or 73 wks)	17	43	Liver	n.r.	14 haemangioendothelioma 34 angiosarcoma 1 HCC 1 hepatocellular adenoma	
						Brown fat	6	6 angiosarcoma	
						Ovary	1	1 angiosarcoma	
						Lung	n.r.	1 angiosarcoma 5 lung adenoma	
						Uterus	1	1 leiomyosarcoma	
						Other	n.r.	1 leukaemia 1 papilloma of the oral cavity	
55	120	18 [(a)]	40 wks + 4 wks control + 11 wks (43, 68 or 73 wks)	12	43	Liver	n.r.	4 haemangioendothelioma 2 hepatocellular adenoma	
						Brown fat	27	27 angiosarcoma	
						Ovary	3	3 angiosarcoma	
						Lung	12	12 lung adenomas	
						Uterus	2	1 sarcoma 1 leiomyoma	
						Auditory gland duct	1	1 squamous cell carcinoma	

STC: sterigmatocystin; b.w.: body weight; n.a.: not applicable; n.r.: not reported; HCC: hepatocellular carcinoma; wks: weeks.
(a): calculated using default values (EFSA, 2012);
(b): Some animals had multiple tumours.

Table D2: Carcinogenicity of sterigmatocystin in rat after oral exposure.

N of animals at beginning/ effective N	Route of application	STC concentration in feed (mg/kg feed)	Daily STC dose (mg/kg b.w.)	Purity (%)	Length of treatment (observation) in weeks	N of animals at end of experiment	First appearance of tumours at week	Organ	N of animals with tumours	Type of tumour/ multiplicity if applicable[(b)]	Reference
Wistar, weanling, 0.05 kg b.w. at start											Purchase and van der Watt (1970a)
10[(a)]	Feeding	0	0	n.a.		9	–	–	0	–	
10[(a)]	Feeding	10 + 15[c]	0.5+0.75[(g)]	88	26 + 26 (123)	9	42	Liver	8	8 HCC 1 hyperplastic nodules 1 invasive fibrosarcoma 1 anaplastic mesenchymal tumour	
10[(a)]	Feeding	20 + 30[c]	1.0+1.5[(g)]	88	26 + 26 (123)	10	42	Liver Not specified	10	10 HCC 1 granulosa-cell tumour	
10[(a)]	Feeding	100 + 150[c]	5.0+7.5[(g)]	88	26 + 26 (123)	3	42	Liver Omentum	3	3 HCC 1 osteogenic sarcoma 1 rhabdomyosarcoma	
10[(a)]	Intragastric 5 days/week	n.a.	0	n.a.	52 (123)	10	–	–	0	–	
10[(a)]	Intragastric 5 days/week	n.a.	0.5[(i)]	88	52 (123)	10	42	Liver	4	4 HCC 2 hyperplastic nodules	
10[(a)]	Intragastric 5 days/week	n.a.	1.0[(i)]	88	52 (123)	9	42	Liver	5	5 HCC 2 hyperplastic nodules	
10[(a)]	Intragastric 5 days/week	n.a.	5.0[(i)]	88	52 (123)	9	42	Liver Spleen	9 1	9 HCC 1 mesenchymal tumour 1 haemangiosarcoma	
Donryu, 6-weeks old, male, 0.12 kg b.w. at start											Ohtsubo et al. (1978)
20/17[(d)]	Feeding	0	0	n.a.	101 (101)	12	0	-	0	-	
20/13[(d)]	Feeding	5	0.25[(g)]	n.r.	101 (101)	3	66	Liver Lung	11 3	1 haemangiosarcoma 2 adenoma 9 well differentiated HCC 6 HCC with tubular pattern 5 poorly differentiated HCC 3 metastasis	
20/13[(d)]	Feeding	10	0.5[(g)]	n.r.	96 (96)	0	66	Liver Lung	12 3	2 haemangiosarcoma 12 well differentiated HCC 5 HCC with tubular pattern 3 poorly differentiated HCC 3 metastasis	
Wistar, male, 4 weeks old											Terao et al. (1978)
30	Feeding	0	0	n.r.	54 (69)	n.r.	n.r.	/	0	/	
15	Feeding	10	0.5[(g)]	n.r.	54 (69)	n.r.	54	Liver	8	8 HCC	

Table D2: Continued.

N of animals at beginning/ effective N	Route of application	STC concentration in feed (mg/kg feed)	Daily STC dose (mg/kg b.w.)	Purity (%)	Length of treatment (observation) in weeks	N of animals at end of experiment	First appearance of tumours at week	Organ	N of animals with tumours	Type of tumour/ multiplicity if applicable[(b)]	Reference
ACI/N, 11 weeks old, male[(h)]											Maekawa et al. (1979)
12/11[e]	Feeding	0	0	n.a.	121 (121)	n.r.	58[(f)]	Testis	4		
								Adrenal gland	1		
								Others	4		
36/27[e]	Feeding	0.1	0.005[(g)]	n.r.	121 (121)	n.r.		Testis	5		
								Adrenal gland	1		
								Others	8		
36/29[e]	Feeding	1	0.05[(g)]	n.r.	122 (122)	n.r.		Liver	1	1 haemangiosarcoma	
								Testis	2		
								Adrenal gland	1		
								Others	3		
36/26[e]	Feeding	10	0.5[(g)]	n.r.	118 (118)	n.r.		Liver	5	1 HCC 3 haemangiosarcoma 3 hyperplastic nodules	
								Testis	4		
								Adrenal gland	2		
								Others	5		

STC: sterigmatocystin; b.w.: body weight; n.r.: not reported; N: number; HCC: hepatocellular carcinoma.
(a): Five male and five female rats.
(b): Some animals had multiple tumours.
(c): Dietary concentration first six months + dietary concentration second six months.
(d): Number surviving at time of appearance of first tumour.
(e): Number of rats surviving 58th week except when autolysis at death was advanced.
(f): Not specified for which dose/control group.
(g): calculated using default values (EFSA, 2012).
(h): incidence of other histological changes in the liver at doses 0, 0.1, 1, 10: hyperplastic foci: 1,4, 9, 21; hyperplastic nodules: 0, 0, 0, 3.
(i): dose reported by the authors as mg/rat (administered 5 days per week; 0.5ml sunflower seed oil as dissolvent or given solely in controls) and converted to mg/kg b.w. based on information from feeding study conducted during the same series of experiments without correction for administration 5 days per week.

Table D3: Overview of carcinogenicity studies of sterigmatocystin in rats and mice after intraperitoneal, dermal and subcutaneous treatment.

Strain	Sex N	Route of application	Daily STC dose	Purity (%)	Length of treatment (observation) (weeks)	N of animals at the end of experiment	First appearance of tumours (weeks)	Organ	N of animals with tumours	Type of tumour/ multiplicity if applicable[(b)]	Reference
Mouse											
CD2F1	M	s.c.		99.9	Single injection (52 weeks)		n.r.				Fujii et al. (1976)
	18		0 mg/kg b.w.			17		Lung	2	2 papillary adenomas[(b)]	
								Liver	1	1 hepatocellular adenoma	
	40		0.5 mg/kg b.w.			36		Lung	4	4 papillary adenomas	
								Liver	6	6 hepatocellular adenoma	
	15		1.0 mg/kg b.w.			14		Lung	3	3 papillary adenomas[(c)]	
								Liver	4	4 hepatocellular adenoma	
								Other	1	1 lymphoma	
	27		5 mg/kg b.w.			23		Lung	9	9 papillary adenomas[(d)]	
								Liver	10	10 hepatocellular adenoma	
	F						n.r.				
	18		0 mg/kg b.w.			18		/	0		
	34		0.5 mg/kg b.w.			34		Lung	1	1 papillary adenomas	
	22		1.0 mg/kg b.w.			22		Lung	2	2 papillary adenomas	
								Submaxillary gland	1	1 adenoma[(e)]	
	33		5 mg/kg b.w.			32		Lung	9	9 papillary adenomas[(c)]	
								Liver	3	3 hepatocellular adenoma	
								Other	1	1 lymphoma	
Rat											
NS	M										Dickens et al. (1966)
	6	s.c. twice/week	0	n.a.	65 (89)	n.r.	/	Skin	1	1 sarcoma-like tumour	
	6	s.c. twice/week	0.5 mg/rat	n.r.	24 (65)	n.r.	47	Skin	3	3 sarcomas	
								Liver		1 HCC 1 cholangioma	

Table D3: Continued.

Strain	Sex N	Route of application	Daily STC dose	Purity (%)	Length of treatment (observation) (weeks)	N of animals at the end of experiment	First appearance of tumours (weeks)	Organ	N of animals with tumours	Type of tumour/ multiplicity if applicable[(b)]	Reference
Wistar	M&F										Purchase and van der Watt (1973)
	10[(a)]	No treatment	No treatment	n.a.	70 (70)	10	/	/	0		
	10[(a)]	Dermal twice/week	0 mg/rat in DMSO	n.a.	70 (70)	10	/	/	0		–
	10[(a)]	Dermal twice/week	0 mg/rat in acetone	n.a.	70 (70)	10	/	/	0		–
	10[(a)]	Dermal twice/week	1mg/rat in DMSO	99	70 (70)	10	40	Skin	10	4 papilloma 6 squamous cell carcinoma	
								Liver	9	5 HCC 4 hyperplastic nodules	
	10[(a)]	Dermal twice/week	1 mg/rat in acetone	99	70 (70)	10	40	Skin	10	3 papilloma 7 squamous cell carcinoma	
								Liver	7	7 HCC	
Wistar	M										Terao (1978)
	30	i.p. once/week	0 mg/kg b.w.[(f)]	n.a.	23 (80)	27	/	/	0		–
	40	i.p. once/week	4 mg/kg b.w.	n.r.	23 (80)	5	28	Liver	1	1 HCC	
								Peritoneum	20	20 mesothelioma	

NS: not specified; i.p.: intraperitoneal; s.c.: subcutaneous; STC: sterigmatocystin; b.w.: body weight; DMSO: dimethylsulphoxide; n.r.: not reported; n.a.: not applicable; HCC: hepatocellular carcinoma; N: number; M: male; F: female.

(a): five male and five female rats.

(b): All except one of the identified lung neoplasia was identified as papillary adenomas. Only one was identified as adenocarcinoma, however the dose and sex was not specified. Therefore the numbers shown for the study by Fujii et al. (1976) should be considered as the number of neoplasia.

(c): One animal with both lung and liver adenomas.

(d): Five animals with both lung and liver adenomas.

(e): In the submaxillary gland.

(f): Vehicle: dimethylformamide.

Appendix E. Benchmark dose analysis

The details of the benchmark dose (BMD) analysis applied to the incidence of hepatocellular carcinomas (HCC) for the intragastric substudy reported by Purchase and van der Watt (1970a) and haemangiosarcomas reported by Maekawa et al. (1979) are presented in this Appendix.

The BMD/L was calculated by means of the software BMDS v2.1.2 (US EPA). All models for dichotomous (quantal) data were selected for the analysis at the default benchmark response (BMR) of 10 % extra risk advised by the EFSA guidance on the use of benchmark dose (EFSA, 2009). Models allowing for restrictions were run only when the fit of the respective unrestricted models would indicate their application after visual inspection of the dose-response data.

Since the BMDL is the lower 95 % one-sided confidence bound of the BMD and the BMDU is the upper 95 % confidence bound of the BMD, the interval BMDL/BMDU represents the 90 % confidence interval of the BMD. This provides a descriptive measure for the accuracy of the BMD, which for an acceptable BMD/L value should not be larger than one order of magnitude (EFSA, 2009).

E.1. BMD analysis of the incidence of hepatocellular carcinomas for the intragastric substudy by Purchase and van der Watt (1970a)

Sterigmatocystin (STC) was administered by gastric intubation at doses of 0, 0.15, 0.3 or 1.5 mg/rat (dissolved in 0.5 mL sunflower seed oil) five days a week for 52 weeks. These doses correspond to 0, 0.357, 0.714, 3.57 mg/kg b.w. per day (based on information from a feeding study conducted during the same series of experiments) after adjusting for the administration of STC on only 5 days per week. Reduced exposure for only 1 year was not adjusted for life-time exposure.

Following intragastric administration of STC 0/10, 4/10, 5/9 and 9/9 animals showed HCC at these four dose groups, respectively (see Appendix D, Table D2).

As shown in Table E1, a range of $BMDL_{10}$ values from different accepted models was observed that substantially exceeded one order of magnitude. In addition large $BMD_{10}/BMDL_{10}$ ratios were obtained for some models. This indicates that the data are not informative enough for determining a reference point (EFSA, 2009, 2011b) and the $BMD_{10}/BMDL_{10}$ values were not further used in the risk assessment.

Table E1: The benchmark dose and the 95 % benchmark dose lower confidence limit values for the incidence of hepatocellular carcinomas reported by Purchase and van der Watt (1970a) calculated for a benchmark response of 10 % extra risk when dosing sterigmatocystin intragastric for 52 weeks.

Models	Restriction	N of parameters	Minus Log-likelihood	P-value	Accepted	BMD_{10} (mg/kg b.w. per day)	$BMDL_{10}$ (mg/kg b.w. per day)
Full model	na	4	12.91	–	–	–	–
Null (reduced) model	-	1	26.29	-	-	-	-
Probit	na	2	14.02	0.33	yes	0.21	0.13
LogProbit	none	2	13.27	0.70	yes	0.16	0.020
Logistic	na	2	14.18	0.28	yes	0.22	0.14
LogLogistic	none	2	13.40	0.61	yes	0.15	0.016
Quantal-Linear	na	1	13.05	0.96	yes	0.080	0.049
Multistage Cancer[(a)]	na	2	13.01	0.91	yes	0.089	0.050
Multistage [(a)]	none	2	13.01	0.91	yes	0.089	0.047
Weibull	none	2	13.05	0.88	yes	0.095	0.0059
Gamma	none	if	if	if R:0.87[(b)]	if R:yes[(b)]	if R:0.092[(b)]	if R:0.049[(b)]

b.w.: body weight; BMD_{10}: benchmark dose for a response of 10 % extra risk; $BMDL_{10}$: 95 % lower confidence limit of the benchmark dose with BMR = 10 % extra risk; na: not applicable, if: invalid fit; N: number
(a): two versions of the multistage model fitted the data with the same model;
(b): R: value obtained when fitting a restricted model.

E.2. BMD analysis of the incidence of haemangiosarcomas reported by Maekawa et al. (1979)

STC was administered via the diet at concentrations of 0, 0.1, 1 and 10 mg STC/kg feed. These concentrations correspond to doses of 0.005, 0.05 and 0.5 mg STC/kg b.w. per day using the default factor of 0.05 recommended by EFSA (2012). No haemangiosarcomas were observed in the control and the 0.005 mg STC/kg b.w. per day dose group, one (3 %) at the 0.05 mg STC/kg b.w. per day dose group and 3 (9 %) at the 0.5 mg STC/kg b.w. per day dose group (see Appendix D, Table D2).

All dichotomous models satisfied the log-likelihood and the goodness-of-fit acceptability criteria when compared to the null model and the full model (Table E2). The BMD_{10} ranged between 0.36 and 0.50 mg/kg b.w. per day and the $BMDL_{10}$ between 0.16 and 0.34 mg/kg b.w. per day. The lowest $BMDL_{10}$ of 0.16 mg/kg b.w. per day was obtained with the log-logistic model (see Figure E1) and was only slightly smaller than that obtained by the multistage model which resulted in the same linear fit as the two other multistage models and the Weibull and Gamma models (see Figure E2).

Table E2: The benchmark doses and the 95 % benchmark dose lower confidence limit values for the incidence of haemangiosarcomas reported by Maekawa et al. (1979) calculated for a benchmark response of 10% extra risk.

Models	Restriction	N of parameters	Minus Log-likelihood	P-value	Accepted	BMD_{10} (mg/kg b.w. per day)	$BMDL_{10}$ (mg/kg b.w. per day)
Full model	na	4	13.65	–	–	–	–
Null (reduced) model	-	1	16.50	-	-	-	-
Probit	na	2	14.36	0.49	yes	0.48	0.33
LogProbit	none	2	14.50	0.19	yes	0.50	0.28
Logistic	na	2	14.40	0.48	yes	0.49	0.34
LogLogistic	none	1	13.99	0.88	yes	0.36	0.16
Quantal-Linear[(a)]	na	1	14.03	0.86	yes	0.36	0.18
Multistage Cancer[(a)]	na	1	14.03	0.86	yes	0.36	0.18
Multistage[(a)]	none	1	14.03	0.86	yes	0.36	0.18
Weibull[(a)]	none	1	14.03	0.86	yes	0.36	0.18
Gamma[(a)]	none	1	14.03	0.86	yes	0.36	0.18

b.w.: body weight; BMD_{10}: benchmark dose for a response of 10 % extra risk; $BMDL_{10}$: 95% lower confidence limit of the benchmark dose with BMR = 10 % extra risk; na: not applicable, N: number.
(a): the three versions of the multistage model and the Weibull and Gamma model fitted the data with the same linear model.

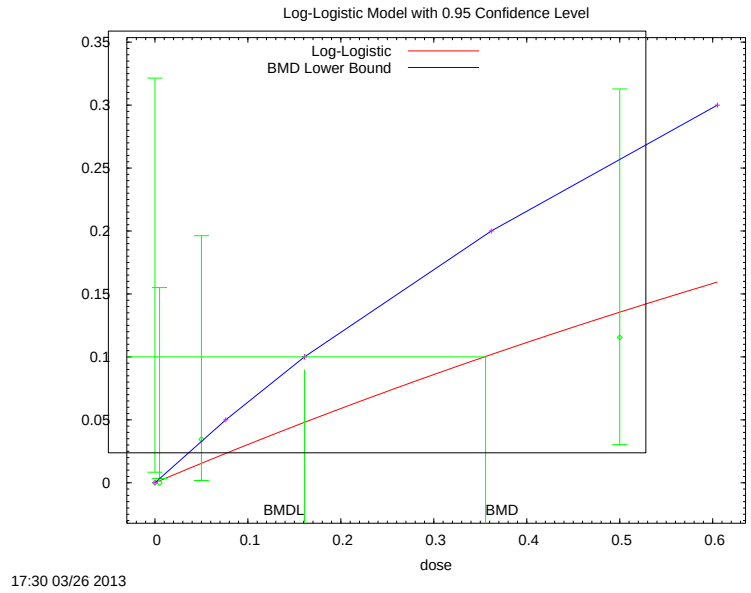


Figure E1: Fit of the Loglogistic Model resulting in a BMD_{10} of 0.36 and a $BMDL_{10}$ of 0.16 mg STC/kg b.w. per day.

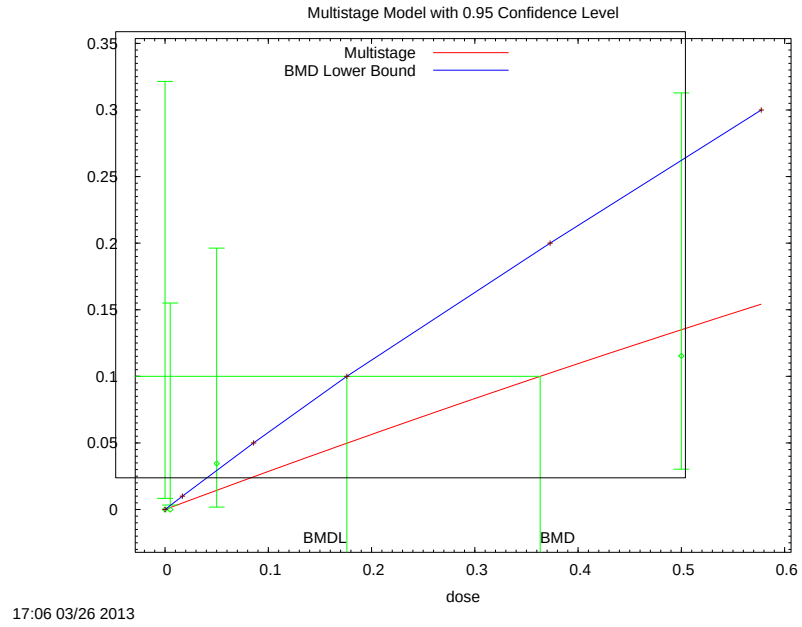


Figure E2: Fit of the Multistage Model, identical to the fit obtained for the other two versions of the Multistage Model as well as for the Weibull and Gamma model, resulting in a BMD = 0.36 and BMDL = 0.18 mg STC/kg b.w. per day.

ABBREVIATIONS

a_w	Water activity
γ-GT	Gamma-glutamyltransferase
ACAT	Acetyl-CoA cholesterol acetyltransferase
AF	Aflatoxin
$AFB_{1,2}$	Aflatoxin $B_{1,2}$
$AFG_{1,2}$	Aflatoxin $G_{1,2}$
AFM_1	Aflatoxin M_1
AFQ_1	Aflatoxin Q_1
AG	Azaguanine
ALT	Alanine aminotransferase
AOAC	Association of Official Analytical Chemists
AST	Aspartate aminotransferase
AVF	Averufin
AVN	Averantin
AVNN	Averufanin
BMD	Benchmark dose
BMD_{10}	Benchmark dose for a response of 10 % extra risk
BMDL	Benchmark dose lower confidence limit
$BMDL_{10}$	The lower 95 % confidence limit for a benchmark response of 10 % extra risk
BMR	Benchmark response
b.w.	Body weight
CHO	Chinese hamster ovary
CI	Confidence interval
CONTAM Panel	EFSA Panel on Contaminants in the Food Chain
CYP	Cytochrome P450
DAD	Diode array detection
DHDMSTC	Dihydrodemethylsterigmatocystin
DHOMSTC	Dihydro-O-methylsterigmatocystin
DHSTC	Dihydrosterigmatocystin
DMSO	Dimethylsulphoxide
DMSTC	Demethylsterigmatocystin
ε	extinction coefficient or molar absorptivity
EFSA	European Food Safety Authority
ELISA	Enzyme-linked immunosorbent assay
ESI^+	Electrospray positive ionization
EU	European Union
FAO	Food and Agriculture Organization
FDA	Food and Drug Administration
FLD	Fluorescence detection
GC	Gas chromatography
GES	Gastric epithelium cell
GLDH	Glutathione dehydrogenase
GSH	Glutathione
HAVN	5-hydroxyaverantin
HCC	Hepatocellular carcinoma
HLA	Human leukocyte antigen
HPLC	High-performance liquid chromatography
HP-TLC	High-performance thin layer chromatography
HPRT	Hypoxanthine–guanine phosphoribosyltransferase
IARC	International Agency for Research on Cancer
IL	Interleukin
i.p.	Intraperitoneal

LC	Left-censored data/results / Liquid chromatography
LC–MS	Liquid chromatography-mass spectrometry
LC–MS/MS	Liquid chromatography-tandem mass spectrometry
LDH	Lactate dehydrogenase
LOD	Limit of detection
LOQ	Limit of quantification
Max	Maximum
Min	Minimum
MLs	Maximum levels
MOE	Margin of exposure
n	Number of samples
n.a.	Not applicable
NER	Nucleotide excision repair
NOR	Norsolorinic acid
n.r.	Not reported
OAVN	Oxoaverantin
OMSTC	O-methylsterigmatocystin
OTA	Ochratoxin A
P50	50th percentile
PBMC	Peripheral blood mononuclear cell
PCNA	Proliferating cell nuclear antigen
pDC	Plasmacytoid dendritic cell
p.o.	Per os
ROS	Reactive oxygen species
s.c.	Subcutaneous
SCE	Sister chromatid exchange
SPE	Solid phase extraction
STC, ST	Sterigmatocystin
TDI	Tolerable daily intake
TG	Thioguanine
TLC	Thin-layer chromatography
TNF-α	Tumour necrosis factor-alpha
UDS	Unscheduled DNA synthesis
UK	United Kingdom
USA	United States of America
UV	Ultraviolet
VAL	Versiconal
VERA	Versicolorin A
VERB	Versicolorin B
VHA	Versiconal hemiacetal acetate
WBC	White blood cell
XP	Xeroderma pigmentosum