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SCIENTIFIC OPINION

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

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

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ABSTRACT

The European Food Safety Authority (EFSA) was asked by the European Commission to deliver a scientific opinion on the health risks from citrinin in food and feed. Citrinin is a mycotoxin produced by several species of the genera *Aspergillus, Penicillium* and *Monascus* and occurs mainly in stored grains. The available occurrence data were not adequate to carry out a dietary exposure assessment. Citrinin is nephrotoxic and a no-observed-adverse-effect level (NOAEL) of 20 µg/kg body weight (b.w.) per day was identified from a 90-day study in rats. Due to the limitations and uncertainties in the database, the derivation of a health-based guidance value was not considered appropriate but a level of no concern for nephrotoxicity of 0.2 µg/kg b.w. per day was determined. Based on the available data a concern for genotoxicity and carcinogenicity could not be excluded at the level of no concern for nephrotoxicity. In the absence of adequate exposure data, characterisation of the risk of citrinin as a food contaminant was based on the estimate of the citrinin concentrations in grains and grain-based products that would result in an exposure equal to the level of no concern for nephrotoxicity. For high consuming toddlers, other children and adults this citrinin concentration is between 9 and 53 µg citrinin/kg and between 19 and 100 µg citrinin/kg for average consumers, respectively. For animals, risk characterisation was based on the estimate of the citrinin concentration in grains that would result in exceedance of the NOAEL of 20 µg/kg b.w. per day for pigs, which ranged between 640 and 1 173 µg/kg. The CONTAM Panel concluded that the impact of uncertainties on the risk assessment is large, and more data regarding the toxicity and the occurrence of citrinin in food and feed in Europe are needed to enable refinement of the risk assessment.

KEY WORDS

Mycotoxins, citrinin, food, feed, dietary exposure, level of no concern for nephrotoxicity, risk assessment

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SUMMARY

The European Food Safety Authority (EFSA) was asked by the European Commission, to deliver a scientific opinion on the risks to human and animal health related to the presence of citrinin in food and feed.

Citrinin is a nephrotoxic mycotoxin produced by several species of the genera *Aspergillus, Penicillium* and *Monascus*. Citrinin is generally formed after harvest and occurs mainly in stored grains, but also in other plant products such as beans, fruits, fruit and vegetable juices, herbs and spices, and also in spoiled dairy products.

Citrinin is known to occur also as an undesirable contaminant in *Monascus* fermentation products (generally described as red mould rice (RMR)), which have been used in Asia for centuries for meat preservation and food colouring.

No previous assessment of citrinin as a food contaminant could be identified. However, in various documents related to the assessment of ochratoxin A, reference is made to the concomitant occurrence of citrinin in food or feed material.

Instrumental techniques for citrinin analysis include fluorimetric, chromatographic and immunochemical techniques. To date, high performance liquid chromatography with fluorescence detection (HPLC-FLD) is the method of choice for routine citrinin analysis. Limits of detection (LOD) as low as 0.1 µg/kg can be achieved. One of the major challenges in citrinin analysis relates to its instability in various organic solvents and at higher temperatures. So far, none of the applied analytical methods has been validated by inter-laboratory studies. In addition, no certified reference materials or proficiency tests are available for the determination of citrinin in food or feed.

Following an EFSA call for data, analytical results from only 30 samples were submitted by one Member State, covering the period between 2006 and 2008. In addition, the Panel on Contaminants in the Food Chain (CONTAM Panel) investigated the occurrence of citrinin in food and feed as reported in the literature. The reported citrinin concentrations varied widely in grains both when intended for food (up to 420 µg/kg) and for feed (up to 998 µg/kg) consumption. For food other than grains, the reported citrinin concentrations varied up to 42 µg/kg in grain-based products, up to 355 µg/kg in herbs and up to 0.2 µg/L in fruit and vegetable juices. The reported citrinin concentrations in feed other than grains also varied widely (up to > 405 µg/kg). In general, a high proportion (up to > 90 %) of left censored data (below LOD or limit of quantification (LOQ)) were observed although not all studies reported LOD or LOQ values. Furthermore, it was noted that information on whether the studies were targeted or non-targeted was mostly missing. In both food and feed, co-occurrence of citrinin with other mycotoxins was observed, especially with ochratoxin A in grains and grain-based products, and with patulin in fruits and fruit and vegetable juices.

The CONTAM Panel concluded that the available occurrence data either submitted to EFSA in response to the call for data or from the literature were not adequate to carry out dietary exposure assessments for either humans or animals.

Citrinin is heat sensitive and decomposes during heat treatment to form other complex compounds, such as citrinin H_1 and citrinin H_2, respectively with higher and weaker cytotoxicity than the original citrinin.

Specific toxicokinetic studies with oral administration are not available for citrinin. Experimental data indicate that citrinin residues may occur in edible tissues and eggs following oral exposure of animals with highly contaminated feed materials.

Acute oral lethal doses (LD_{50}) in mice and rabbits are of the order of 100 mg/kg body weight (b.w.).

In repeat dose toxicity studies, the kidney was identified as the principal target organ for citrinin and significant species differences in the susceptibility to citrinin have been observed. An 80-week study in rats, with a high dietary exposure to citrinin (1 000 mg/kg feed), revealed general mild morbidity and progressive histopathological changes and prominent adenomas in the kidneys. Studies of the immunotoxicity of citrinin are rather incomplete and often non-specific and do not allow a conclusive evaluation.

In vitro and *in vivo* studies provided clear evidence for reproductive toxicity and teratogenic and embryotoxic effects of citrinin. The doses tested in the *in vivo* experiments exerted, however, clear signs of maternal toxicity including nephrotoxicity, indicating that these effects might be secondary to maternal toxicity. One study in male mice showed adverse effects on male reproductive organs and sperm quality when citrinin was given intraperitoneally.

Published data suggest that citrinin is not mutagenic in conventional bacterial assays, with or without metabolic activation, but induces micronuclei, aneuploidy and chromosomal aberrations *in vitro* in mammalian cells. *In vivo* it induced chromosome abnormalities and hypodiploidy in the bone marrow of mice. Available animal studies revealed that citrinin induces renal adenomas. However as no life-time exposure studies are available, no conclusion can be drawn regarding the potential carcinogenicity of citrinin.

From the data available on mammalian cells in culture, the CONTAM Panel concluded that citrinin toxicity is exerted via multiple pathways such as DNA and RNA synthesis inhibition, inhibition of microtubule assembly and of tubulin polymerization, alteration of mitochondrial functionality with consequent increase in reactive oxygen species (ROS) production, inactivation of the heat shock protein 90 (HSP90) multichaperone complex and activation of the signal transduction pathway and the caspase-cascade system that result in apoptotic cell death.

The available evidence indicates that citrinin at low doses does not exacerbate the toxic effects of other mycotoxins. The CONTAM Panel concluded that the combined effect of citrinin and ochratoxin A is at most additive.

A 90-day toxicity study in rats showed no adverse effects at a citrinin dose of 20 µg/kg b.w. per day in feed, the highest dose tested in this study. The CONTAM Panel concluded that this study is suitable to identify a no-observed-adverse-effect level (NOAEL) of 20 µg citrinin/kg b.w. per day for nephrotoxicity. However, this study did not provide any information on dose-response since at all doses tested, no adverse effects were observed.

Given the limitations and uncertainties in the current database on citrinin, the CONTAM Panel concluded that the derivation of a health based guidance value was not appropriate. For compounds that may be genotoxic and carcinogenic, EFSA recommends the use of a margin of exposure (MOE) approach for risk characterisation. However, due to the lack of data on human dietary exposure, no MOE could be calculated. In order to give risk managers a tool to evaluate the risk of citrinin in food and feed, the Panel decided to characterise the risk of citrinin on the available data on nephrotoxicity and determined therefore a level of no concern for nephrotoxicity. Applying a default uncertainty factor of 100 to the NOAEL of 20 µg/kg b.w. per day, accounting for inter-species variation and for inter-individual variation, the CONTAM Panel concluded that there would be no concern for nephrotoxicity in humans at an exposure level of 0.2 µg/kg b.w. per day. Based on the available data, a concern for genotoxicity and carcinogenicity could not be excluded at the level of no concern for nephrotoxicity.

For humans, in the absence of adequate exposure data, characterisation of the risk of citrinin as a food contaminant was based on the estimate of the critical citrinin concentrations in grains and grain-based products that would result in an exposure equal to the level of no concern for nephrotoxicity. Consumption of grains and grain-based products was selected for this approach because the majority of the analytical results relate to citrinin concentrations in grains. Moreover, grain and grain-based

products are a common major commodity in human foods. Nevertheless, there is evidence that food commodities other than grains and grain-based products can also be sources of citrinin, but the overall contribution to human exposure could not be estimated. For high consuming toddlers, other children (from 3 up to and including 9 years of age) and adults the critical citrinin concentration was in the range between 9 and 53 µg/kg grains and grain-based products and between 19 and 100 µg/kg grains and grain-based products for average consumers of these age classes. Based on the available data no firm conclusion can be made regarding the likelihood of exceeding the level of no concern for nephrotoxicity on a daily basis over a prolonged period.

Regarding the toxicity of citrinin in target animal species, only a few studies on adverse effects of citrinin in pigs, rabbits, poultry and dogs could be identified. No effect was reported in a study with pigs given 20 µg citrinin/kg b.w. per day. The CONTAM Panel considered this intake value as a NOAEL, which is consistent with the results from a subchronic study in rodents. In poultry, the reported effects varied widely in type and severity depending on species, age of the birds and the design of the studies. As further details were missing, these results were not suitable for risk characterisation and no NOAEL/lowest-observed-adverse-effect level (LOAEL) for poultry could be identified. In rabbits, moderate health effects were reported when citrinin was administered via the feed. However, no intake value could be calculated and no NOAEL/LOAEL for rabbits could be identified. The available data for dogs could not be used for risk assessment due to co-exposure with ochratoxin A. No other data for companion animals could be identified. Experimental data regarding systemic toxic effects in ruminants were not available. It is assumed that citrinin is highly degraded and metabolised through the microbial activity in the forestomachs of ruminants. However, an impairment of the rumen flora due to the antibacterial effect of citrinin cannot be excluded.

For animals, risk characterisation was based on the estimate of critical citrinin concentrations in grains that would result in an exposure equal to the identified NOAEL. There is evidence that feed commodities other than grains can also be sources of citrinin, but the overall contribution to animal exposure could not be estimated. The critical citrinin concentration resulting in an exposure equal to the NOAEL is between 640 and 1 173 µg/kg grains for pigs. The CONTAM Panel concluded that it is unlikely that pigs will consume grains that exceed the critical citrinin concentration on a daily basis over a prolonged time. The limited data available did not allow for a risk characterisation for other animals than pigs.

The CONTAM Panel evaluated the impact of uncertainties on the derived critical citrinin concentrations in food and feed. Due to the lack of exposure data, lack of sufficient toxicological dose-response information and limited information on the mode of action, the CONTAM Panel concluded that the magnitude of the overall uncertainties is large, and that the risk characterisation remained incomplete.

Therefore, there is a need for more data regarding the occurrence of citrinin in food and feed in Europe as well as for certified reference materials and defined performance criteria for the analysis of citrinin in food and feed. There is also a need for well-designed toxicological studies in laboratory animal species to further explore the toxicological potential of citrinin and to characterize the dose-response relationships. Furthermore, more data are needed on farm animal toxicity and the carry over of citrinin from the feed to animal products intended for human consumption.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

A number of Penicillium species, including *P. verrucosum*, have been reported to produce citrinin. Also some *Aspergillus* species are reported to produce citrinin. Because these fungi are also major producer of ochratoxin A and aflatoxins in grains, co-occurrence of citrinin with ochratoxin A and aflatoxin B_1 has been found in grains, particularly rice. Also the simultaneous occurrence of patulin and citrinin in apple juices and apple jams has been reported.

According to some scientific publications, citrinin is acutely nephrotoxic at relatively high doses in mice and rats, rabbits, pigs and poultry, causing swelling and eventual necrosis of the kidneys and affecting the liver function at a lesser extent; The International Agency for Research on Cancer (IARC) has reviewed the available data and concluded that there is limited evidence for carcinogenicity in animals[4]. It has also been reported that citrinin may be implicated in human disease, such as "yellow rice" disease in Japan and Balkan Endemic Nephropathy (BEN) when present with other mycotoxins, in particular OTA.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

In accordance with Art. 29 (1) (a) of Regulation (EC) No 178/2002 the Commission asks EFSA for a scientific opinion on the risks to human and animal health related to the presence of citrinin in feed and food.

The scientific opinion as regards the presence of citrinin in feed and food should, *inter alia,* comprise the:

a) evaluation of the toxicity of citrinin for humans, considering all relevant toxicological endpoints;

b) exposure of the EU population to citrinin, including the consumption patterns of specific (vulnerable) groups of the population (e.g. high consumers, children, people following a specific diet, etc).

c) determination of the daily exposure levels of citrinin for the different farm animal species (difference in sensitivity between animal species) above which

- signs of toxicity can be observed (animal health / impact on animal health) or
- the level of transfer/carry over of citrinin from the feed to the products of animal origin for human consumption results in unacceptable levels of citrinin.
- identify feed materials which could be considered as sources of contamination by citrinin and the characterisation, insofar as possible, of the distribution of levels of contamination for the different (groups of) feed materials.

d) assess the co-occurrence of citrinin with ochratoxin A and/or aflatoxins and/or patulin in feed and food

[4] http://monographs.iarc.fr/ENG/Monographs/vol40/volume40.pdf

ASSESSMENT

1. Introduction

1.1. General information

Citrinin is a polyketide mycotoxin produced by several species of the genera *Aspergillus*, *Penicillium* and *Monascus* (see Appendix A). Some of the citrinin producing fungi are also able to produce the mycotoxins ochratoxin A or patulin. Citrinin is generally formed after harvest under storage conditions and it occurs mainly in grains, but can also occur in other products of plant origin e.g. beans, fruits, fruit and vegetable juices, herbs and spices and also in spoiled dairy products. In addition, citrinin is found as an undesirable contaminant in red mould rice (RMR) which is used as a food preservative and colourant in Asian foods (Fink-Gremmels et al., 1991).

During research for antibiotic agents in the middle of the last century, interest in citrinin arose when its broad antibacterial activity was identified (Raistrick and Smith, 1941). However, interest decreased when its mammalian toxicity was demonstrated (Ambrose and DeEds, 1946).

1.2. Previous assessments

No previous assessments could be identified. However, in various documents on the assessment of ochratoxin A, reference is made to the concomitant occurrence of citrinin in a given food or feed material.

1.3. Chemistry

Citrinin is a polyketide mycotoxin [$C_{13}H_{14}O_5$, IUPAC: (3*R*, 4*S*)-4,6-dihydro-8-hydroxy-3,4,5-trimethyl-6-oxo-3*H*-2-benzopyran-7-carboxylic acid; molecular weight 250.25 g/mol; CAS No: 518-75-2] (Figure 1). It forms acidic lemon-yellow crystals with maximal ultraviolet (UV) absorption at 250 nm and 333 nm (in methanol), melting at 175 °C with decomposition. Citrinin crystallizes in a disordered structure, with the *p*-quinone and *o*-quinone tautomeric forms in a dynamic equilibrium in the solid state (Poupko et al., 1997). Citrinin has a conjugated, planar structure which gives its natural fluorescence (the highest fluorescence is produced by a non-ionized citrinin molecule at pH 2.5, Franco et al., 1996). Citrinin is practically insoluble in cold water but soluble in aqueous sodium hydroxide, sodium carbonate, or sodium acetate; in methanol, acetonitrile, ethanol, and most other polar organic solvents (Xu et al., 2006).

Besides citrinin, a large number of citrinin derivatives have been isolated from different fungal species in search of antitumor compounds indicating that citrinin might be a precursor of novel active compounds (e.g. pennicitrinones A-C, pennicitrinols A-B, dicitrinones A-C (Wakana et al., 2006; Lu et al., 2008; Du et al., 2010)).

Several studies have been carried out on degradation of citrinin revealing that decomposition of citrinin occurs at > 175 °C under dry conditions, and at > 100 °C in the presence of water. Known decomposition products include citrinin H_2 which did not show significant cytotoxicity, while the decomposition product citrinin H_1, which is made up of two citrinin molecules (Figure 1), showed an increase in cytotoxicity as compared to the parent compound (Trivedi et al., 1993a; Trivedi et al., 1993b; Hirota et al., 2002; Xu et al., 2006). Another decomposition product, the cytotoxic citrinin dimer, dicitrinin A, was also reported in 2006, together with other monomeric and dimeric degradation products (Clark et al., 2006).

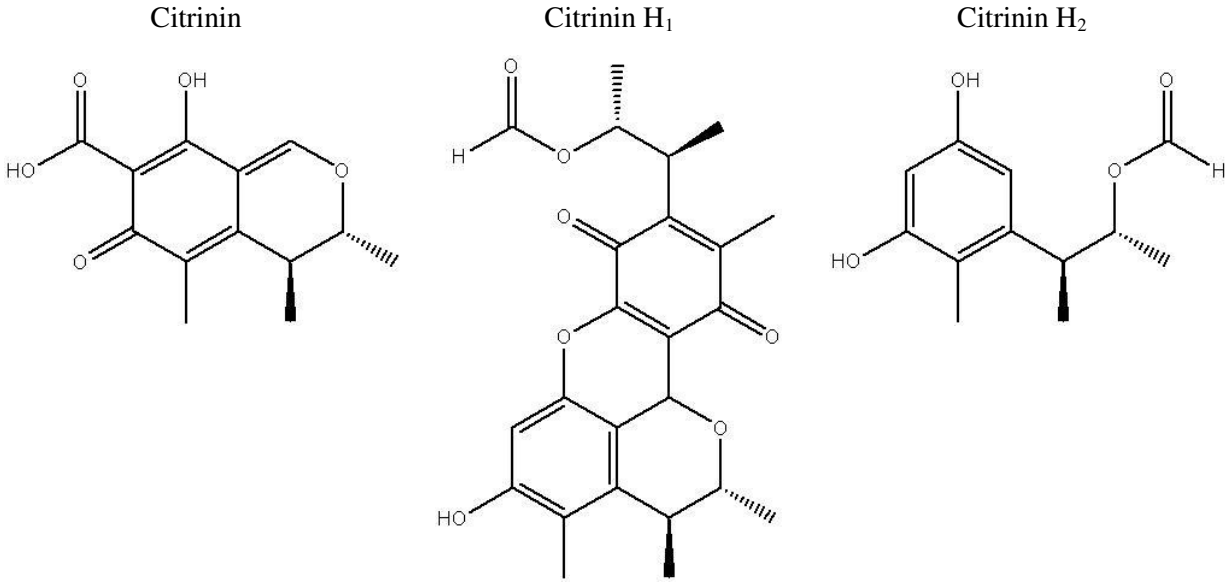

Figure 1: Chemical structures of citrinin and decomposition products citrinin H_1 and citrinin H_2.

2. Legislation

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

No MLs have been reported by the Food and Agriculture Organization (FAO) for citrinin in food and feed (FAO, 2004).

3. Methods of sampling and analysis

A review of the qualitative and quantitative analytical methods for citrinin published from 1980 to early 2004 was performed by Xu et al. in 2006. After that date, no updated review has been published.

Citrinin analysis has been done with single analyte methods, but also together with ochratoxin A (Lepom, 1986; Vazquez et al., 1996; Molinié et al., 2005; Polisenska et al., 2010), and in multi-mycotoxin methods (Spanjer, 2007; Tangni et al., 2009; Rasmussen et al., 2010). So far, none of the methods mentioned in this section have been validated by inter-laboratory studies. Citrinin reference standards can be obtained from several commercial suppliers, but no certified reference materials or proficiency tests are available.

[5] 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.
[6] 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.
[7] 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.

One of the major challenges in citrinin analysis relates to its instability (see Section 1.3) depending on temperature, solvent compositions used for sample preparation, mobile phase and preparation of standard citrinin solution (Xu et al., 2003).

3.1. Sample collection and storage

Fungal growth and mycotoxin production during storage are generally 'spot processes' significantly affected by crop variety, agronomic practices and, weather conditions during harvest, post harvest drying and cleaning, storage and processing conditions as well as toxigenic potential of the mould species. Consequently, the distribution of mycotoxins in a lot of agricultural products is heterogeneous ('hot spots'). Thus, sampling is the largest source of variability associated with mycotoxin analysis and the most crucial step in obtaining reliable results (Köppen et al., 2010). Sampling methods are available for the official control of mycotoxins in grains and grain-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 citrinin.

3.2. Sample preparation

The most frequently reported method for sample preparation for grains and derived products, is extraction with chloroform-based solvent partition. Several modifications were performed to the time-consuming protocol in order to improve recovery rates (Lepom, 1986; Molinié et al., 2005; Dohnal et al., 2010; Polisenska et al., 2010). The highest reported recoveries varied between 77 and 83 % (Dohnal et al., 2010).

Liquid-liquid extraction with a diatomaceous-earth adsorbent (Abramson et al., 1999) and extraction with dichloromethane with addition of phosphoric acid, and polyamide column clean-up (Meister, 2004) resulted in recoveries between 70 and 90 %. Extraction with methanol/water (70/30, v/v) and immunoaffinity purification was performed with different food samples (Sato et al., 2010). Recoveries between 60 and 120 % were reported. Commercial immunoaffinity columns (IAC) exist for citrinin. Hartl and Stenzel (2007) used aminopropyl solid phase extraction (SPE) columns after ethyl acetate extraction of cereal samples and RMR. These authors tested several unhalogenated solvents for extraction (including methanol, toluene and acetonitrile), but only ethyl acetate achieved acceptable citrinin recoveries in the range of 77 to 92 %. The use of aminopropyl SPE resulted in a better separation of citrinin from matrix compounds.

Zheng et al. (2009) discussed the presence of citrinin in *Monascus* products at the outside as well as the inside of fungi cells. Extraction with ethanol solution only extracted the citrinin outside the cell. A new extraction method involving ultrasonic treatment was proposed. Ultrasonic treatment disrupts cells, promoting the extraction of citrinin (Zheng et al., 2009).

New developments for sample preparation include a molecularly imprinted polymer (MIP) developed by Guo et al. (2010). 1-Hydroxy-2-naphthoic acid was used as mimic template for the MIP design. Preparation of rice samples before MIP solid phase extraction was very simple (ultrasonic extraction with methanol/water (7/3, v/v), centrifugation and filtration). Recoveries obtained were in the range of 86 to 99 %.

3.3. Instrumental techniques for quantification

Instrumental techniques for citrinin analysis include colorimetric, chromatographic and immunochemical techniques. Table 1 summarises the characteristics and the limits of detection (LOD) of the different analytical methods.

[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.

1. Colorimetric techniques that are based on the natural fluorescence of citrinin are generally susceptible to diverse interfering factors and therefore characterized by low recoveries and high LODs (100-8000 µg/kg) (Trantham and Wilson, 1984; Vazquez et al., 1997). Due to the high LODs they are no longer used.

2. Chromatographic techniques:

a. Thin-layer chromatography (TLC) was frequently used in mycotoxin analysis, and also in citrinin analysis, before high performance liquid chromatography (HPLC) became widespread. TLC methods were reviewed by Xu et al. (2006) for the period 1980-2004. They were applied to apples, grains, fungal isolates and beers. Most of them were only used for qualitative analysis, while the quantitative ones had rather high LODs (15-40 µg/kg).

b. HPLC has been successfully applied to the analysis of citrinin in grains, fungal cultures, cheese, feeds, dietary supplement RMR and biological fluids. Detection is performed by fluorescence (FLD) or UV, including photodiode-array (PDA) detection, while reversed phase (RP) chromatography is mostly used. LODs range from 0.1-10 µg/kg in different food matrices. In general, reversed phase (RP)-HPLC-FLD methods afford relative good sensitivity and recovery, but application of these methods to various complex food and feed matrices is time-consuming. Lengthy clean-up procedures are generally needed.

3. Chromatographic techniques coupled to mass spectrometry

a. Liquid chromatography mass spectrometry (LC-MS) for citrinin was reported for the first time by Tuomi et al. (2001). The method included RP-HPLC separation with MS identification and quantification using electrospray ionization (ESI) in the positive mode on an ion trap mass analyser. To date, LC-MS methods are preferred for simultaneous analysis of a very broad range of mycotoxins in different food and feed samples. However, only a limited number of these methods include citrinin (see below) (Spanjer et al., 2008; Rasmussen et al., 2010).

b. Gas chromatography mass spectrometry (GC-MS) can be applied if mycotoxins are sufficiently volatile at the column temperature or can be converted into volatile compounds by derivatisation. Shu and Lin (2002) described the quantitative analysis of citrinin in *Monascus* products by GC-selected ion monitoring (SIM)-MS without the need for chemical derivatisation.

4. Immunochemical techniques have been developed for many mycotoxins as a rapid alternative to chromatography. These methods can be highly specific since they are based on antibody-antigen interactions. However, cross-reactivity with structurally related compounds is possible depending on the applied antibody. Therefore, positive immunoassay results should be confirmed by another analytical technique such as HPLC. For citrinin, this problem of cross-reactivity seems to be less relevant. Although the quantification might be prone to a substantial uncertainty, immunochemical methods clearly give an indication of the presence of the analyte. Being rapid and low cost, some of these methods are very useful as screening methods and they are used by many control and research laboratories.

a. Enzyme linked immunosorbent assays (ELISA) for citrinin detection have been reported in wheat, barley, maize, RMR, and other grains, with LODs ranging from 2 to 15 000 µg/kg (Abramson et al., 1995, 1996, 1999; Heber et al., 2001; Hartl and Stenzel, 2007; Kononenko and Burkin, 2007; Duan et al., 2009; Li et al., 2010).

b. An electrochemical immunosensor incorporated in a microfluidic cell for quantification of citrinin in rice samples was developed by Arevalo et al. (2011). Measurements were done by amperometry. The electrochemical detection could be done within 2 min and the total assay

time was 45 min, which is more rapid than chromatographic methods. The LOD was 0.1 µg/L. Other advantages are the small volumes of reagents used. The immunoreactor developed can operate as a fast, selective, and sensitive detector when it is incorporated into a flow injection analysis system.

In recent years, focus in mycotoxin analysis has moved from single-analyte detection towards multi-mycotoxin or even multi-analyte detection. Wu et al. (2011) described a rapid, synchronous analytical method for the determination of citrinin, and several bioactive compounds (monascin, ankaflavin, lactone and acid forms of monacolin K) in RMR. These compounds were extracted by the same method without clean-up, separation was done by RP-HPLC and detected by PDA (for the bioactive compounds) connected to FLD (for citrinin) or by mass spectrometry. Kokkonen et al. (2005) developed a RP-HPLC-PDA method for the simultaneous analysis of ten mycotoxins including citrinin in different fungal culture media. Zheng et al. (2009) developed an HPLC-FLD-PDA-MS method for chemical fingerprint profiling of *Monascus* products including detection of different pigments and citrinin. Tabata et al. (2008) developed a LC tandem MS (MS/MS) method for the simultaneous determination of ochratoxin A, ochratoxin B and citrinin. The limit of quantification (LOQ) for citrinin was 0.1µg/kg. Thirty-three mycotoxins were simultaneously extracted with an acetonitrile/water mixture, diluted with water and then directly injected into the LC-MS/MS system. But the LOD was high for citrinin (100 µg/kg in pistachio and maize slurry) (Spanjer et al., 2008). Rasmussen et al. (2010) described an LC-MS/MS method for the detection of 27 mycotoxins in maize silage. Extraction was based on a very fast and simple method for analysis of multiple pesticide residues in food known as QuEChERS (Quick Easy Cheap Effective Rugged Safe). Validation results for citrinin were unsatisfactory. Removal of more matrix components or the use of more acidic eluents was proposed to improve citrinin detection when using this method. However, these examples clearly show that a quantification of citrinin together with other mycotoxins when using multi-mycotoxin methods is still problematic and needs further research.

Table 1: Overview of characteristics and reported limits of detection of most important citrinin analytical methods.

Analytical technique	Method characteristics	Limits of detection (µg/kg)
Colorimetric techniques	Screening (qualitative – semi-quantitative)	100 - 8 000
Thin-layer chromatography	Screening (qualitative – semi-quantitative)	15 - 40
Enzyme linked immunosorbent assay	Screening (semi-quantitative – quantitative)	2 - 15 000
High-performance liquid chromatography with fluorescence or photo-diode array detection	Confirmation (semi-quantitative – quantitative) Possible multi-analyte detection	0.1-10
Liquid chromatography mass spectrometry	Confirmation (semi-quantitative – quantitative) Possible multi-analyte detection	0.1-100

3.4. Concluding comments

Sample preparation is the key issue for the detection of citrinin. Rigorous clean-up methods are needed to obtain methods with low LODs, but at the same time need to take into account the limited stability of citrinin to some organic solvents. To date, HPLC-FLD is the method of choice for routine citrinin analysis. LODs as low as 0.1 µg/kg can be achieved (Table 1).

4. Occurrence of citrinin in food and feed

4.1. Occurrence of citrinin in food and feed: call for data

In October 2010, EFSA launched a call for data on several contaminants, including citrinin, with a closing date of January 2011. Subsequent to this call, EFSA received and evaluated the results on citrinin reported from the analysis of 30 samples. The data submission to EFSA followed the

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

4.1.1. Summary of data collected

Data on citrinin occurrence (n = 30) were received from only one Member State. Two thirds of the analytical results were obtained on samples collected in 2008 and the remaining samples from 2006. All samples were collected in the framework of national programmes on targeted samples.

Data were classified according to FoodEx, which is a food classification system developed by EFSA's Dietary and Chemical Monitoring (DCM) Unit in 2009 with the objective of simplifying the link between chemical occurrence and food consumption data when assessing the dietary exposure to hazardous substances (EFSA, 2011a).

Analytical results were obtained only from samples belonging to the food group "Grains and grain-based products". In particular, three samples were classified in the food sub-group of "Grains for human consumption" (barley and wheat grains) and 27 samples in the sub-group of "Unprocessed grains of undefined end-use" (hereafter referred as "Unprocessed grains"). Since their end-use is not established, and grains for human consumption normally undergo several processing steps before consumption, it has been considered as not appropriate to include them in the human exposure assessment.

The reported analytical method used to perform the analyses of citrinin was HPLC-FLD. All results were reported on whole weight and corrected for recovery. For food matrices like "Grains and grain-based products", the LOD and LOQ reported were 0.5 and 1.5 µg/kg, respectively. In 23 out of 30 samples, citrinin was not detected and in three samples, the concentration of citrinin was reported as non-quantified. In only four samples of "Unprocessed grains", citrinin was quantified at 1.8, 5.2, 13.2 and 93.6 µg/kg, respectively. These concentrations of citrinin are within the range of those reported in the literature, as summarised below in Table 2.

The Panel on Contaminants in the Food Chain (CONTAM Panel) considered that the data set was too limited to assess human dietary exposure since most of the samples analysed (27 out of 30 samples) were not specifically for human consumption, and the remaining samples classified for human consumption were all below the LOD and LOQ. In addition, they represented a limited data set from only one member state.

4.2. Previously reported literature data on the occurrence of citrinin

Due to the very limited data set EFSA obtained from the call for data, an in-depth analysis of the available literature was performed. Compared to ochratoxin A, little attention has been paid to citrinin, and the natural occurrence is consequently described in only a small number of publications. It should be noted that not all studies reported LOD and LOQ values, meaning that negative findings must be interpreted with caution. In the case of positive samples, the impact of targeted versus non-targeted sampling could not be assessed.

4.2.1. Occurrence of citrinin in grains

A detailed overview of published occurrence studies of citrinin in grains is given in Appendix B, Table B1.

One of the first comprehensive studies on the occurrence of citrinin was done by Scott et al. (1972) in Canada in 1968. Grain samples associated with lung problems in farmers and silo operators were collected from farm storages and analysed. The grain had been stored under damp conditions, resulting in heating and spoilage. After development of an appropriate screening method, 13 of 29 samples were found to contain citrinin (0.07 to 80 mg/kg). The contaminated samples included wheat, oats, barley and rye. All samples positive for citrinin were also contaminated with ochratoxin A.

In Europe studies have been mainly carried out in South-Eastern European countries, where the occurrence of citrinin has been linked in the past to the so-called Balkan endemic nephropathy (BEN). Vrabcheva et al. (2000) monitored incidence of ochratoxin A and citrinin in cereal samples intended for use as food and feed from villages in Bulgaria where BEN had occurred. Samples were analysed for citrinin using an ELISA with an LOD of 5 µg/kg. Two of the 3 citrinin-positive wheat samples (37 samples) were also positive for ochratoxin A, and citrinin concentrations were two to 200 times higher than those of ochratoxin A. A maximum citrinin concentration (420 µg/kg) was found in a wheat sample intended for human consumption having also the highest ochratoxin A content (39 µg/kg). Citrinin and ochratoxin A were not detected in barley (6 samples) intended for feed use. Also oats (n = 9) intended for feed use did not contain citrinin in detectable amounts, although ochratoxin A was detected at concentrations up to 140 µg/kg. Maize (n = 23) was found to be free of ochratoxin A and citrinin. Another study (Petkova-Bocharova et al., 1991) carried out 10 years earlier with samples from a BEN region in Bulgaria, and using TLC for citrinin determination (LOD 15 - 20 µg/kg), reported a contamination frequency for citrinin in stored maize from endemic and nonendemic areas of 27-44 % and 10-15 %, respectively. The concentrations of citrinin ranged from 50 to 1500 µg/kg for endemic and from 50 to 380 µg/kg for nonendemic areas.

Polisenska et al. (2010) analysed 11 wheat samples (for food use) from the Czech Republic shortly after harvest. There was only one sample positive for citrinin, which had a low content not exceeding the LOQ (1.5 µg/kg). The same sample had an ochratoxin A content of 4.7 µg/kg. The authors also analysed three barley samples destined for malt production. One of the samples was offered to a malt house but not accepted due to a higher content of admixtures and impurities and a mouldy smell. This sample contained the highest citrinin content (93.6 µg/kg) and also contained ochratoxin A (31.4 µg/kg). Barley (n = 6) and wheat (n = 11) for feed use were also analysed by these authors and citrinin was found in only 3 barley samples (up to a concentration of 13.2 µg/kg).

Kononenko and Burkin (2008) detected citrinin in grains for feed use (LOD = 10 µg/kg). Wheat (n = 43), barley (n = 138) and maize (n = 157) contained citrinin in 5 %, 4 % and 2 % of the samples, with maximum values of 144, 998, and 953 µg/kg respectively. Samples of wheat (n = 25) and maize (n = 30) for animal consumption, that were collected in 1997 from Western Romania were analysed for mycotoxin contamination by Curtui et al. (1998). Citrinin was only found in one maize sample at a concentration of 580 µg/kg.

In Western European countries, studies on the occurrence of citrinin have been carried out in Switzerland and the United Kingdom (UK). In Switzerland, Dick et al. (1988) found citrinin in two out of four samples of durum wheat with a concentration of 0.3 and 0.7 µg/kg (LOD = 0.1 µg/kg). In the United Kingdom, 48 of 141 cereal samples for feed use were found to be contaminated with citrinin (Scudamore and Hetmanski, 1995; Scudamore et al., 1997) reporting a maximum of 10 µg/kg (LOD = 1µg/kg).

In Asia, Nishijima (1984) investigated 27 samples of grains for food use taken in the Tokyo metropolitan area and did not detect citrinin. In India where maize is the third most important crop, Janardhana et al. (1999) collected 197 maize grain samples and, using TLC, found citrinin in only one sample, with a citrinin concentration of 12 µg/kg. In Japan, Tabata et al. (2008) investigated citrinin in grains for food use with LC-MS/MS. Citrinin was detected in one wheat sample at a concentration of 0.19 µg/kg, together with ochratoxin A, and in two buckwheat samples at concentrations of 0.55 and 0.62 µg/kg, also with ochratoxin A.

In six out of 18 parboiled rice samples from Andhra Pradesh, India, Reddy et al. (1983) found citrinin with concentrations ranging from 12 to 55 µg/kg. No citrinin was detected in maize (n = 30), sorghum (n = 20), ragi (n = 37) or broken rice (n = 32). Nguyen et al. (2007) investigated the occurrence of 3 mycotoxins (aflatoxin B_1, citrinin and ochratoxin A) in rice samples (n = 100) collected from 5 provinces of the central region of Vietnam, using HPLC with fluorescence detection (LOD = 0.11 and LOQ = 0.35 µg/kg). Citrinin was detected in 13 % of the samples at concentrations up to

0.42 µg/kg. These samples were collected during the rainy season. No citrinin was found in rice in the dry season.

The occurrence of citrinin has also been reported in grains from Africa. El-Sayed (1996) found citrinin in 56 % of barley samples (n = 27) in Egypt with an average concentration of 64.4 µg/kg, in 8.3 % of yellow maize samples with an average concentration of 62.9 µg/kg, and in 39 % of rice samples with an average concentration of 13.8 µg/kg. Citrinin was not detected in wheat samples. In the study of Odhav and Naicker (2002) none of the grain samples (n = 30) for beer production in South Africa contained citrinin. Aziz et al. (2006) found citrinin in 5 out of 70 samples of grains at concentrations from 100 to 300 µg/kg. Citrinin was detected in 10 out of 30 samples of rice in Egypt in concentrations between 2.74 and 28.54 µg/kg (Abd-Allah and Ezzat, 2005).

Although the available information from the literature is scarce and reflects citrinin concentrations from studies performed for different purposes (targeted as well as non-targeted sampling), the CONTAM Panel summarised citrinin occurrence values that were obtained with HPLC, ELISA and LC-MS in Table 2. These methods were selected based on their quantitative characteristics. The summary shows that detected citrinin concentrations in grains vary widely in Europe when intended for human consumption (up to 420 µg/kg food, n = 79) and when intended for animal consumption (up to 998 µg/kg feed, n = 766). In grains, citrinin often co-occurs with ochratoxin A (see for further details Appendix B, Table B1).

Table 2: Summary of the occurrence of citrinin in grains obtained from published studies carried out across Europe, Asia and Africa and analysed with HPLC, ELISA or LC-MS. Details are listed in Appendix B, Table B1.

	n[(a)]	% LC[(b)]	Descriptive statistics (µg/kg feed or food)	
			Min[(c)]	Max[(d)]
Europe				
Grains intended for human consumption	79	90	0.1	420
Grains intended for animal consumption	766	91	0.5	998
Asia				
Grains intended for human consumption	131	88	0.11	0.62
Africa[(e)]				
Grains of undefined end use[(f)]	70	93	n.r.	300

n: number of samples, LC: left censored data (values below the LOD or LOQ), n.r. not reported, Min: minimum, Max: maximum.
(a): Total number of samples analysed with HPLC, ELISA or LC-MS for the specific matrix and the specific continent, see also Appendix B, Table B1.
(b): Percentage of left censored data among the total number of analytical results that are included in Appendix B, Table B1 and that were obtained with HPLC, ELISA or LC-MS for the specific matrix (feed or food) and the specific continent.
(c): The lowest LOD value reported across literature studies carried out with HPLC, ELISA or LC-MS, see also Appendix B, Table B1.
(d): The highest citrinin concentration across literature studies carried out with HPLC, ELISA or LC-MS, see also Appendix B, Table B1.
(e): Not specified whether the grains will be used for human consumption or animal consumption.
(f): Only data from Egypt analysed with HPLC, ELISA or LC-MS were available.

4.2.2. Occurrence of citrinin in food other than grains

The Appendix B, Table B2 gives a detailed overview of previously reported occurrence studies in food other than grains.

4.2.2.1. Grain-based products

As citrinin occurs in grains, it is also found in grain-based products. Osborne (1980) used a TLC method to detect citrinin in mouldy bread (n = 50) and mouldy flour (n = 7) in England. Citrinin was

only detected in one flour sample but at a concentration that was too low to be quantified. Dick et al. (1988) found citrinin in 11 out of 21 flour samples, with a concentration range of 0.2-1.0 µg/kg. One of two pasta samples contained citrinin at a concentration of 0.5 µg/kg, however none of the wheat bran samples (n = 5) contained citrinin. Meister (2004) showed that citrinin was especially detectable in grain-based products such as brans, wheatings and shorts, containing a higher ratio of the outer layers of the grain kernel. Molinié et al. (2005) described a methodology for simultaneous extraction/purification of ochratoxin A and citrinin with a recovery for citrinin of 80 % and an LOD of 0.5 µg/kg. They confirmed that for breakfast cereals, if citrinin was present its content was higher than that of ochratoxin A. They analysed 45 samples, of which 31 samples (69 %) were contaminated with ochratoxin A at 0.2-8.8 µg/kg, and 8 samples (18 %) were contaminated with citrinin in the range of 1.5 to 42 µg/kg. When citrinin was found in a sample, it always occurred with ochratoxin A. The sample with the highest citrinin concentration (42 µg/kg) contained ochratoxin A at a concentration of 4.1 µg/kg.

Nishijima (1984) tested 31 samples of commercial flour which were marketed in Tokyo and detected 27 and 73 µg/kg citrinin in two samples of maize flour. No citrinin was detected in maize starch (n = 1), maize soup (n = 3) or maize flakes (n = 2). As both positive maize samples were sold by the same distributor, more samples from this distributor were analysed and concentrations up to 1 390 µg/kg were detected.

Besides wheat and other grains, El-Sayed analysed Egyptian wheat flour (n = 31) but no detectable amounts of citrinin were found (El-Sayed, 1996). In Ghana, Kpodo et al. (1996) found citrinin in all 20 fermented maize dough samples with concentrations from 0.7 µg/kg to 585 µg/kg (LOD = 0.1 µg/kg).

4.2.2.2. Beans and peas

Petkova-Bocharova et al. (1991) reported contamination of beans samples from the BEN region in Bulgaria (LOD = 5-20µg/kg), with a frequency for citrinin of 27 to 40 % (depending on the sampling year) for endemic (with concentrations 20-1000 µg/kg) and 10 to 12 % (depending on the sampling year) for non endemic areas (with concentrations 20-200 µg/kg).

Aziz et al. (2006) detected citrinin in one out of ten samples of Egyptian kidney beans at a concentration of 370 µg/kg. Nishijima (1984) did not detect citrinin in beans in Japan (n = 4), nor El-Sayed in beans or peas from Egypt in 42 and 32 samples, respectively (El-Sayed, 1996).

4.2.2.2. Fruits, fruit juices and vegetable juices

Apples with rotten spots can contain citrinin alone, or co-occurring with patulin. The fruit disease is caused by strains of *P. expansum* and the mould growth occurs mainly where the surface tissue of fruit has been damaged. Infection commonly follows insect or storm damage during preharvest, rough gathering at harvest or strong washing and sorting procedures after harvest.

Ciegler et al. (1977) reported that cultures of *P. expansum* isolated from apples produced both patulin and citrinin. Vinas et al. (1993) studied the citrinin producing capacity of 122 *P. expansum* isolates from apples in Spain and verified that 46 % of those examined produced citrinin in a culture medium (glucose yeast agar) and 73 % of the producers were isolated from decayed apples.

One hundred samples of apples were collected from households in Croatia (Pepeljnjak et al., 2002). Among Penicillia isolates, 78 % (29 isolates) were *P. expansum*. Citrinin was detected in 19 % of apple samples, in concentrations ranging from 50 to 240 µg/kg. In a study of Gimeno and Martins (1983) 5 out of 30 naturally rotted apples were contaminated with aflatoxin B_1 and citrinin at 20 - 200 µg/kg and 300-3000 µg/kg, respectively, and 3 apples were contaminated with citrinin at 1 000-2 500 µg/kg (LOD = 30-40 µg/kg). Later Martins et al. (2002), using the same rapid multidetection TLC method (LOD = 15-20 µg/kg), analysed 351 apples with different-sized brown rotten areas from markets in Portugal for patulin and citrinin. Out of these samples 3.9 % contained

only citrinin and 19.6 % contained both citrinin and patulin. The mean citrinin concentrations were in the range from 320 to 920 µg/kg. Andersen et al. (2004) have not detected citrinin in 6 samples of naturally infected fruits, juices and pulps.

Dietrich et al. (2001) analysed fruit (apple, cherry, black currant and grape) and vegetable (tomato) juices sampled from retail stores in Germany using ELISA (LOD = 0.08 µg/kg) and traces of citrinin (maximum 0.2 µg/L) could be found in 4 out of 55 samples.

Canadian researchers (Harwig et al., 1973) reported the occurrence of patulin and citrinin as well as patulin-producing strains of *P. expansum* in naturally rotten, stored, and windfall apples from Ontario and Nova Scotia. Citrinin was present at detectable concentrations in 2 out of 61 apples.

Besides in apples, citrinin has been detected (LOD = 40 -100 µg/kg) in 2 grape samples (both at 70 µg/kg), one fig sample (60 µg/kg) and one pear sample (50 µg/kg) (Aziz and Moussa, 2002).

4.2.2.3. Other products

In 1974, Subramanyam and Rao reported the occurrence of citrinin in Indian groundnuts infected with *A. flavus*, *P. citrinum* and *A. terreus* (Subramanyam and Rao, 1974). In a study of Jimenez et al. (1991) 168 samples of roasted nuts (almonds (*Prunus dulcis*), peanuts (*Arachis hypogea*), hazelnuts (*Corylus avellana*), pistachio nuts (*Pistacia vera*), and sunflower seeds (*Helianthus annuus*) were investigated for mycotoxins and the presence of mycotoxin producing fungi. The results of the study showed that these commodities were rarely contaminated with mycotoxins and were negative for citrinin. This may be related to the low moisture level generally present in these products. However, in all samples, there was a microflora capable of developing toxins when cultivated in appropriate media. The same negative results for citrinin were obtained in a study of Abdel-Gawad and Zohri (1993) from the analysis of almonds (n = 5), cashew nuts (n = 5), chestnuts (n = 5), hazelnuts (n = 5), pistachio nuts (n = 5) and walnuts (n = 5) collected from different markets in Saudi Arabia. Also in Egypt, citrinin was not detected in hazelnuts (n = 20), walnuts (n = 20), peanuts (n = 10) and coffee beans (n = 20) (Abdel-Hafez and Saber, 1993; El-Sayed, 1996; Aziz et al., 2006).

In the UK, Jarvis (1983) investigated 44 mouldy commercial cheeses and 17 of them contained citrinin up to 50 µg/kg, as well as ochratoxin A (up to 260 µg/kg). Ochratoxin A and citrinin were not found (LOD = 20 µg/kg) in a German survey of 49 cheeses from various countries (Nowotny et al., 1983).

Santos et al. (2009) screened 84 samples of aromatic and/or medicinal herbs sold in Spain, using an ELISA (LOD = 16.5 µg/kg) and found 61 % of samples contaminated with citrinin (up to 355 µg/kg in ginkgo leaves). In two samples of ginkgo leaves with the highest concentrations of citrinin contamination, the toxin co-occurred with ochratoxin A, aflatoxin B_1, zearalenone, T-2 toxin and deoxynivalenol. Roy and Kumari (1991) collected seed samples (n = 60) of 6 medicinal plants known to have curative properties for various human diseases, from different storage centers in India. Eleven samples were contaminated with citrinin at a concentration between 10 and 760 µg/kg.

Saxena and Mehrotra (1989) reported on the natural occurrence of citrinin in different spices commonly marketed in India. Contaminated spices were turmeric (2 out of 9 with citrinin concentrations of 48 and 52 µg/kg), coriander (1 out of 9 with a citrinin concentration of 34 µg/kg), fennel (2 out of 9 with citrinin concentrations of 28 and 59 µg/kg), black pepper (1 out of 8 with a citrinin concentration of 50 µg/kg), cardamom (1 out of 6 with a citrinin concentration of 25 µg/kg) and cumin (1 out of 8 with a citrinin concentration of 22 µg/kg). El-Kady et al. (1995) investigated a total of 120 different samples belonging to 24 kinds of spices collected from different places at Assiut Governorate (Egypt) for the natural occurrence of mycotoxins. TLC analysis of spice extracts revealed the presence of citrinin in two samples of black cumin at concentrations of 8 and 12 µg/kg.

In India, Kumari and Nusrath (1987) collected from markets 384 samples of coconut products, including dry copra, copra-meal, coconut candy and coconut oil, during different seasons for the evaluation of natural contamination by mycotoxins. They found 4 samples (1 %) to be contaminated

with citrinin of which three samples were dry copra (20-60 µg/kg) and one copra-meal (10 µg/kg). In two dry copra samples citrinin was found in combination either with aflatoxin B_1 or with ochratoxin A.

A study for the contamination of black olive samples (n = 10) from Morocco with mycotoxins was reported by El Adlouni et al. (2006). All olive samples contained ochratoxin A ranging from 0.5 µg/kg to 1.02 µg/kg, 80 % of olive samples contained citrinin between 0.2 and 0.5 µg/kg (LOD = 0.2 µg/kg) and 40 % of the tested samples contained aflatoxin B_1 above 0.5 µg/kg. These data were in line with those of Heperkan et al. (2006) who found citrinin in 34 of the 42 samples (81 %) from the Marmara Region in Turkey and the amount of citrinin varied from a minimum of 75 to a maximum of 350 µg/kg. In the Aegean Region 20 of the 27 (74 %) samples contained citrinin with the highest value being 100 µg/kg. There was a large difference between the citrinin amounts in the two regions of Turkey, with the Marmara Region having much higher concentrations (Heperkan et al., 2006).

Maize-based alcoholic beverages were examined in South Africa where maize is frequently contaminated by mycotoxin-producing fungi and mycotoxins, but none of the beer samples (n = 35) contained citrinin (Odhav and Naicker, 2002). Cerutti et al. (1987) also did not find citrinin in 24 beer samples imported in Italy from 11 European countries (LOD = 1 µg/kg).

A summary of the above described occurrence studies on citrinin in food other than grains obtained via HPLC, ELISA or LC-MS analysis is shown in Table 3. These methods were selected based on their quantitative characteristics. From the available data it was clear that only a scarce database is present in Europe, but also in other continents. Within Europe, the citrinin concentrations found in grain-based products vary up to 42 µg/kg (n = 73). In grain-based products, citrinin often co-occurs with ochratoxin A. In medicinal and aromatic herbs concentrations are reported up to 355 µg/kg (n = 84). Citrinin also occurs in fruits, fruit juices and vegetable juices, where concentrations up to 0.20 µg/L have been observed (n = 61). In fruit, citrinin often co-occurs with patulin, as shown by TLC analysis. In medicinal and aromatic herbs, co-occurrence of mycotoxins is observed. Further details regarding co-occurrence are given in Appendix B, Table B2.

Table 3: Summary of occurrence of citrinin in foods other than grains obtained from published studies carried out across Europe, Asia and Africa and analyzed with HPLC, ELISA or LC-MS. Details are listed in Appendix B, Table B2.

	n[(a)]	% LC[(b)]	Descriptive statistics (µg/kg food)	
			Min[(c)]	Max[(d)]
Europe				
Grain-based products	73	73	0.1	42
Fruits, fruit juices and vegetable juices	61	93	0.08	0.2
Medicinal and aromatic herbs	84	39	16.5	355
Cheeses	93	82	20	50
Africa				
Grain-based products	20	0	0.7	585
Beans and peas	10	90	n.r.	370
Nuts	10	100	n.r.	n.a.
Black olives	10	20	0.2	0.5

n: number of samples, LC: left censored data (values below the LOD or LOQ, n.a.: not applicable, n.r.: not reported, Min: minimum, Max: maximum.

(a): Total number of samples that are analysed with HPLC, ELISA or LC-MS for the specific matrix and the specific continent, see also Appendix B, Table B2.

(b): Percentage of left censored data among the total number of analytical results that are included in Appendix B, Table B2 and that are obtained with HPLC, ELISA or LC-MS for the specific matrix and the specific continent.

(c): The lowest LOD value reported or the lowest citrinin concentration reported (in case of 0 % LC) across literature studies carried out with HPLC, ELISA or LC-MS, see also Appendix B, Table B2.

(d): The highest citrinin concentration across literature studies carried out with HPLC, ELISA or LC-MS, see also Appendix B, Table B2.

4.2.3. Occurrence of citrinin in feed other than grains

Citrinin-producing mould species are generally classified as storage fungi, invading feed commodities at the post-harvest stage. Besides grains for feed use, also other stored feed samples have been analysed for their citrinin content. The Appendix B, Table B3 gives a detailed overview of previously reported occurrence data in feed.

Talmaciu et al. (2008) studied the occurrence of citrinin in feed samples originating from industrial and family-owned farms from Romania in 2007. All samples contained citrinin in the range 17 to > 405 µg/kg with 25 % of the samples containing more than 405 µg/kg. Feed samples from pig and chicken farms in Bulgaria that had reported incidences of nephropathy in the livestock (enlarged and mottled or pale appearance of kidneys at slaughter time) were analysed for their mycotoxin content in 2006 and 2007. Citrinin was found in 92 % and 96 % of the samples, respectively with mean concentrations of 54.7 ± 27.5[9] µg/kg and 120.5 ± 43.3 µg/kg. Besides citrinin, also other mycotoxins were observed, including ochratoxin A (in 2006: 188.8 ± 27.3 µg/kg and in 2007: 376.4 ± 63.9 µg/kg), penicillic acid (in 2006: 838.6 ± 223.9 µg/kg and in 2007: 904.9 ± 86.5 µg/kg), fumonisin B_1 (in 2006: 5564.1 ± 584.4 µg/kg and in 2007: 3254.5 ± 480.6 µg/kg), deoxynivalenol (in 2006: 72.7 ± 18.8 µg/kg and in 2007: 51.4 ± 8.5 µg/kg), penitrem A (in 2006: 1840.4 ± 243.8 µg/kg and in 2007: 713.9 ± 88.2 µg/kg) and zearalenone (in 2006: 133.2 ± 15.5 µg/kg and in 2007: 108.2 ± 9.9 µg/kg) (Stoev et al., 2010). Vrabcheva et al. (2000) analysed 24 Bulgarian wheat bran samples (LOD = 5 µg/kg) of which 5 contained citrinin (5 to 230 µg/kg) and ochratoxin A. Kononenko and Burkin (2008) detected citrinin in feed samples and ingredients (LOD = 10 µg/kg). Out of 829 compounded feeds 8.8 % were positive for citrinin with concentrations in the range of 12 to 182 µg/kg. The highest incidence (28.9 %) was found for sunflower oil-seed meal and cakes with concentrations in the range of 14-397 µg/kg. Also soy-bean samples (2 %), maize gluten samples (16 %) and one wheat bran sample (3 %) contained citrinin at concentrations in the range 14-62 µg/kg.

Scudamore et al. (1997) analysed animal feed from the UK in 1992. Rice bran, maize products, cottonseed meal, rapeseed, sunflower, olive pulp, palm products, soya, peas and beans, manioc and

[9] Mean ± standard error of the mean.

citrus pulp (LOD = 5-20 µg/kg) were analysed. Citrinin was detected in one sample of palm kernel meal at a concentration of 7 µg/kg, together with ochratoxin A and aflatoxins, and in one sample of peas/beans at a concentration of 9 µg/kg together with ochratoxin A.

In France three studies were carried out on the occurrence of mycotoxins in maize silage for dairy cattle. It was observed that the highest citrinin concentrations occurred at the bottom of the silage (average concentration up to 36.6 ± 2.3 µg/kg dry matter). In addition to citrinin, deoxynivalenol, aflatoxin B_1, gliotoxin and zearalenone were also detected (Garon et al., 2006; Richard et al., 2007; 2009).

No studies on the occurrence of citrinin in pet foods could be identified.

From the available data, it was clear that only a scarce database is present in Europe. Based on the quantitative characteristics of the used methods, only studies carried out with HPLC, ELISA or LC-MS were selected for the determination of citrinin in feed other than grains. These studies (n = 1582 with 84 % left censored data) show that the detected citrinin concentrations in feed vary widely in Europe up to > 405 µg/kg feed. In feed, co-occurrence of mycotoxins was observed, which was also confirmed by other studies (e.g. Monbaliu et al., 2010) (for further details see Appendix B, Table B3).

4.2.4. *Monascus* products

A recent concern is the presence of citrinin in food colourings traditionally made in Asia from rice fermented with *Monascus* spp., known as red mould rice (RMR). Synonyms for RMR are red yeast rice, red fermented rice, anka or ang-kak.

M. purpureus and its fermentation products have been used as a food colorant, flavour enhancer, for meat preservation and wine brewing in the Orient for centuries. Recently they have also been used as dietary supplements because of their cholesterol-lowering ability, blood pressure-lowering effects and antioxidant effects. However, during the past 10 years, some researchers have discovered that some *Monascus* species produce citrinin. Blanc et al. (1995a, b) demonstrated that one of the pigments produced by *Monascus* is identical in structure to citrinin. This was the first report of the occurrence of citrinin among metabolites produced by *Monascus*.

Dietrich et al. (1999a) developed an ELISA method for citrinin with an LOD of 2 µg/kg. This method was applied to analyse 12 vegetarian "meat-like" products and 11 Asian foods purchased in retail stores in Bavaria. Citrinin was found in 9 vegetarian "meat-like" products and in one Asian food in a concentration range of 9 to 79 µg/kg. According to the authors all positive findings could be attributed to the use of rice fermented by *Monascus* spp. for food colouring. Furthermore, citrinin concentrations of 2800 µg/kg and 157 µg/kg were detected by HPLC analysis in 2 samples designated as natural food colorants. Sabater-Vilar et al. (1999) detected citrinin in all commercial *Monascus* preparations (12 samples) collected in The Netherlands originating from China in concentrations ranging from 200 to 17 100 µg/kg. Heber et al. (2001), using an enzyme immunoassay (LOD = 15 mg/kg), found citrinin in 7 out of 9 Chinese RMR dietary supplements at measurable concentrations, while Gordon et al. (2010) used LC-MS/MS (LOD not reported) and found in 4 out of 12 commercial RMR formulations citrinin in the concentration range 24 to 189 mg/kg.

Liu et al. (2005) measured the citrinin concentrations in commercial *Monascus* products collected in Taiwan using HPLC analysis. All six *Monascus* products examined contained citrinin at concentrations ranging from 280 to 6 290 µg/kg. Citrinin was only detected in the lipid extracts and not in the aqueous extracts of these products due to its low water solubility.

In the interpretation of these results it needs to be considered that many of these traditional *Monascus* products are only used in very small amounts as food colorants. The intentional use of *Monascus* products as food additives is not authorized in the EU and hence not addressed in this Opinion.

4.2.5. Conclusion

The number of investigations dealing with occurrence of citrinin is small relative to studies concerning other mycotoxins, and the available occurrence data are consequently limited. However, citrinin has been detected in many different feed- and foodstuffs, most often in grains and grain-based products, and has been reported as a natural contaminant in Canada, USA, Africa, Asia and various European countries. In some cases citrinin has been found in trace amounts only, whereas in other cases concentrations up to 998 µg/kg have been measured. Citrinin occurs frequently together with other mycotoxins. In grains and grain-based products, co-occurrence with ochratoxin A is noted. In some reports the concentration of citrinin is several times higher than that of the accompanying ochratoxin A. In fruit, patulin is most often found together with citrinin. In medicinal and aromatic herbs, and feed, co-occurrence with several other mycotoxins is noted.

4.3. Food and feed processing

There is little information on the fate of citrinin during processing. No specific studies on the influence of feed processing on citrinin were identified.

Jackson and Ciegler (1978) demonstrated that citrinin is heat sensitive by evaluating the influence of sterilization of fermented maize on the citrinin content. Autoclaving resulted in the biggest reduction but even heating at 70 °C for an extended period of time resulted in the loss of citrinin.

Kitabatake et al. (1991) investigated the heating conditions used for food cooking (frying and baking) and food processing (extrusion cooking) on citrinin. They found that at up to 150 °C the appearance of citrinin did not change; by heating at and above 160 °C the yellow citrinin became brown. In dry heating the decomposition of citrinin started at 160 °C, and at 175 °C the compound was completely decomposed. Also Betina (1989) observed that citrinin melts and decomposes at 175 °C. An interesting observation of Kitabatake et al. (1991) was that, under semi-moist conditions, the decomposition and detoxication temperatures were both reduced by about 35 °C compared with those under dry heat. On the contrary, Lee et al. (2007) did not observe a reduction of the citrinin concentration of RMR during dry-heating. Below 140 °C no significant change of the citrinin concentration was observed while an increase was noted at 175 °C. Also under wet heating conditions, the citrinin concentration increased when RMR was heated at 120 and 140 °C compared to 90 °C. However at 175 °C a decrease was observed.

Trivedi et al. (1993b) found that heating citrinin in the presence of water at 100 ºC for 30 min caused formation of citrinin H_1 (see Figure 1). Heating at 140-160 °C immediately converted 20 % of citrinin to citrinin H_2 (see Figure 1) (Hirota et al., 2002). Heating at higher temperature or for a prolonged time did not increase the amount of citrinin H_2. The authors suggested that citrinin H_2 decomposed further to form other complex compounds. Citrinin H_2 did not show significant cytotoxicity to HeLa cells in contrast to citrinin H_1. However, no studies analysing citrinin H_1 and/or H_2 in heated food or feed commodities could be identified.

Kpodo et al. (1996) concluded that fermentation did not appear to affect citrinin concentrations but cooking of fermented maize dough seemed to destroy citrinin.

Krogh et al. (1974) noted that citrinin added to mash degraded at a faster rate than ochratoxin A during the mashing process and was not present in detectable amounts in the wort. Similarly, citrinin was also destroyed during the mashing step and, therefore, was not prevalent in beer (Scott, 1996; Odhav and Naicker, 2002).

The possibility of citrinin being masked by conjugation with major food components (e.g. proteins, hydrocarbons, fat) has not been investigated.

In conclusion, citrinin is thermolabile in aqueous solutions above 70 °C. Its decomposition in food and feed depends on temperature and moisture content. Known decomposition products include citrinin H_1

and H_2. Whereas H_2 shows weaker cytotoxicity compared to citrinin, H_1 shows a more pronounced toxic effect. Published data on the stability and the presence of H_1 and H_2 in food or feed were not identified.

5. Food and feed consumption

5.1. Food consumption

5.1.1. EFSA's Comprehensive European Food Consumption Database

In 2010, the EFSA Comprehensive European Food Consumption Database (Comprehensive Database) was built from existing national information on food consumption. Competent authorities in the European countries provided EFSA with data from the most recent national dietary survey in their country at the level of consumption by the individual consumer.

Thus, for the present assessment, food consumption data were available from 28 different dietary surveys carried out in 17 different European countries as follows:

1. Infants: 2 countries; 2 dietary surveys
2. Toddlers: 7 countries; 9 dietary surveys
3. Other children: 13 countries; 17 dietary surveys
4. Adolescents: 10 countries; 12 dietary surveys
5. Adults: 14 countries; 15 dietary surveys
6. Elderly: 7 countries; 7 dietary surveys
7. Very elderly: 6 countries; 6 dietary surveys

Within the dietary studies, subjects were classified in different age classes as defined below:

1.	Infants:	< 12 months old
2.	Toddlers:	≥ 12 months to < 36 months old
3.	Other children:	≥ 36 months to < 10 years old
4.	Adolescents:	≥ 10 years to < 18 years old
5.	Adults:	≥ 18 years to < 65 years old
6.	Elderly:	≥ 65 years to < 75 years old
7.	Very elderly:	≥ 75 years old

In particular, results from consumption surveys from 13 different European countries for children gathered by means of the EFSA Article 36 project 'Individual food consumption data and exposure assessment studies for children' (acronym EXPOCHI) (Huybrechts et al., 2011) were incorporated in the database. Consumption records were codified according to the FoodEx classification system, which has been developed by the DCM Unit in 2009 (EFSA, 2011a).

Detailed information on the 32 dietary surveys included in the Comprehensive Database can be found in the recently published guidance on the "Use of the EFSA Comprehensive European Food Consumption Database in exposure assessment" (EFSA, 2011b). Overall, the food consumption data gathered at EFSA in the Comprehensive Database are the most complete and detailed data currently available in the EU. However, consumption data were collected by using different methodologies and thus they are not suitable for direct country-to-country comparison.

5.1.2. Food consumption data for specific age classes

The existing information on occurrence of citrinin in food suggests that relatively high citrinin concentrations can be found in grains and grain-based products. Following this observation and considering the high consumption of cereal-based foods in the general population, the CONTAM Panel considered that grains and grain-based products might be the major contributor to dietary exposure to citrinin. Average and high consumption (95th percentile) of grains and grain-based

products in the total population were retrieved from all European dietary surveys and summarised in Appendix C (data retrieved from the Comprehensive Database[10]). The percentage of consumers of this food group covers almost 100 % of the subjects in all dietary surveys.

As retrieved from the Comprehensive Database, the 95th percentile consumption of grains and grain-based products in the general population is 12 g/kg body weight (b.w.) per day for infants, 19 g/kg b.w. per day for toddlers, 23 g/kg b.w. per day for other children, 12 g/kg b.w. per day for adolescents, 7 g/kg b.w. per day for adults, 6 g/kg b.w. per day for elderly and 7 g/kg b.w. per day for very elderly.

5.2. Feed consumption

In contrast to the situation for the human population (see Section 5.1), there is no comprehensive database on feed consumption by livestock in the EU. In an attempt to provide a comparable risk characterisation for various animal species, the CONTAM Panel uses general estimates of feed intakes based on EFSA (2009), while the maximum inclusion levels of grains in feed are based on Ewing (1997) and B.M. Paragon (2011, personal communication). These data are used subsequently (see Section 8.2) for risk characterisation. However, in this assessment a detailed consumption analysis was performed only for species for which sufficient data for a risk characterisation were available, namely pigs (see Section 7.4).

Approximately 150 million tonnes of compound feeds are produced in the EU each year. In addition to compound feeds, livestock consume grains and legumes produced on-farm; although no official figures are available, these probably represent an equivalent volume. At least 60 % of all grains produced in the EU are used in livestock feeds (FEFAC, 2009).

The amounts of feed consumed by livestock are influenced by many factors, of which the size, type and age of the animal, and the level of productivity, are particularly important. The choice of feed will be determined by the feeds available and their cost, and their suitability in meeting the nutritional needs of the animal (McDonald et al., 2011).

In attempting to summarise the feed consumption of livestock, it must be stressed that there is considerable variation in feeding systems throughout Europe and that the examples given do not represent 'average' diets, nor do they necessarily reflect 'typical' feeding systems applicable to all production systems in the Europe. Instead, they are used to estimate levels of exposure to citrinin that might not be atypical.

5.2.1. Pigs

There is a considerable range of pig production systems in Europe and of diets fed. However, the majority are characterised by feeding systems in which pigs for fattening are fed cereal-based diets supplemented with vegetable proteins (e.g. soybean meal, peas and beans, sunflower and rapeseed meal). For breeding pigs, the relative proportions of these ingredients in the diets will be different during pregnancy and lactation. Diets for breeding pigs also tend to include greater proportions of fibrous feeds such as cereal by-products and sugar beet pulp (McDonald et al., 2011).

Table 4 shows feed intakes proposed by EFSA (2009) and maximum inclusion levels for grains proposed by Ewing (1997) for piglets, fattening pigs (body weight range 20-100 kg) and lactating sows (200 kg b.w.).

[10] http://www.efsa.europa.eu/en/datex/datexfooddb.ht

Table 4: Live weights and feed intake for pigs (EFSA, 2009) and maximum inclusion level for grains in feed proposed by Ewing, 1997.

	Live weight (kg)	Feed intake (kg dry matter per day)	Maximum inclusion level for grains in feed (%)
Pigs: piglets	20	1.0	55
Pigs: fattening pigs	100	3.0	50
Pigs: lactating sows	200	6.0	50

6. Exposure assessment in animals and humans

6.1. Human exposure assessment of citrinin

The CONTAM Panel considered that the occurrence data on citrinin submitted to EFSA were not adequate to carry out a dietary exposure assessment for the general population or specific population groups including vegetarians.

Therefore, occurrence data of citrinin from the literature (see Section 4.2) in Europe were reviewed for suitability to assess human exposure. Occurrence of citrinin in European countries has been published for different foodstuffs like grains and grain-based products, fruits, aromatic and medicinal herbs, but most studies report on grains and grain-based products. Nevertheless, due to the limited number of studies and the lack of detailed information, the CONTAM Panel concluded that even the available occurrence data in the literature were not adequate to carry out a dietary exposure assessment for the general population or specific population groups.

The CONTAM Panel identified one previous human exposure assessment (Pfohl-Leszkowicz et al., 2007). A total diet study was made for 28 days in 15 families from the Balkan region, but citrinin analysis was only performed for three families. Citrinin was detected in the pooled food on 3 days (10.7 %) for one family, on 6 days (21.4 %) for the second family and not detected for the third family. No measured concentrations in the pooled food were reported and hence the interpretation of these results remains incomplete and they were not used for human exposure assessment in this opinion.

In the absence of relevant occurrence data on food that would allow an exposure assessment, the CONTAM Panel decided to use the consumption data of grains and grain-based products for the calculation of a critical citrinin concentration in this food group that would result in an exposure equal to the level of no concern for nephrotoxicity (see Section 7.6). This critical citrinin concentration needs to be considered as an average concentration over a prolonged period of exposure. Consumption of grains and grain-based products were used for this approach because the majority of the reported occurrence data on citrinin relates to this food group. Moreover, as presented in Section 5.1.2, grains and grain-based products are widely consumed foods in the total population. The results of the calculation of this critical citrinin concentration in the food group of grains and grain-based products are presented in Section 8.1.

6.1.1. Non-dietary exposure

Exposure to mycotoxins through inhalation and skin contact can occur in indoor environments. However, the extent of possible health hazards caused by inhaled mycotoxins or through dermal exposure of mycotoxins is largely unclear (Mayer et al., 2007).

Literature data are scarce on natural occurrence of citrinin in indoor environments. Tuomi et al. (2000) did find citrinin in indoor materials. They analysed 79 bulk samples of mouldy interior surfaces for 17 mycotoxins in Finnish buildings having moisture problems, by means of LC-MS/MS. The collected building materials included wallpaper, cardboard, wood, plasterboard, sand, soil, linoleum, polyurethane insulation, and paint. Three of the 79 samples were contaminated with citrinin in a

concentration range between 20 and 35000 ng/g (fresh weight) of sample. But also other mycotoxins such as sterigmatocystin, satratoxins, diacetoxyscirpenol, deoxynivalenol, verrucarol, and T-2-tetraol were present.

In conclusion, multi-mycotoxin contaminations including citrinin, in indoor environments via inhalation or dermal contact may contribute to the total human exposure.

6.2. Animal exposure assessment of citrinin

No previous animal exposure assessments could be identified. In addition, suitable occurrence data were not reported by European countries to EFSA. Therefore, the available scientific literature on the occurrence of citrinin in feed in European countries was reviewed (see Section 4.2) for different feedstuffs like grains and compound feeds.

The CONTAM Panel noted that except in a few studies, detailed information was missing, including for example the target animals of the investigated feed materials. Due to these limitations, the CONTAM Panel concluded that the occurrence data were not adequate to estimate dietary animal exposure to citrinin.

In the absence of relevant occurrence data on feed that would allow an exposure assessment, the CONTAM Panel decided to use the data on consumption of grains intended for feed for the calculation of a critical citrinin concentration in grains that would result in an exposure equal to the no-observed-adverse-effect level (NOAEL) (see Section 7.4). This critical citrinin concentration needs to be considered as an average concentration during a prolonged period of exposure. Consumption of grains was used for this approach, because the majority of the analytical results relate to citrinin concentrations in grains. Moreover, grains are a common major commodity in animal feeds; although other feed commodities can also be sources of citrinin, there are insufficient data on concentrations in these feeds to permit exposure assessment. The critical citrinin concentration was calculated for pigs, the only species for which sufficient data for a risk characterisation were available, as described in Section 8.2.

7. Hazard identification and characterisation

7.1. Toxicokinetics

7.1.1. Experimental animals

Specific toxicokinetic studies with oral administration are not available for citrinin. Citrinin is eliminated predominantly by renal excretion, as described in a study with radiolabelled citrinin (^{14}C-citrinin) by Reddy et al. (1982) in which approximately 75 % of the i.p. dose was recovered in urine. ^{14}C-citrinin was administered to pregnant female rats by subcutaneous injection of a dose of 35 mg/kg b.w. on the 12th day of gestation. Elimination of ^{14}C-citrinin-derived radioactivity from plasma was found to be biphasic. The half-lives for the rapid (alpha) and slower (beta) phases of elimination were 1.95 h and 39.7 h, respectively. Approximately 74 % of the radioactivity appeared in the urine in the first 24 h, with only 1.7 % and 1.4 % in the urine at 48 h and 72 h, respectively.

Sándor et al. (1991) described a subacute toxicity study with ochratoxin A and citrinin in pigs. In this limited study, groups of three animals were given citrinin, ochratoxin A or both compounds and compared with a group of five control animals. Citrinin was given at a dose of 0.02 mg/kg b.w. once or daily for a period of 8 weeks. The combination group of three animals received both citrinin (0.01 mg citrinin/kg b.w.) and ochratoxin A (0.01 mg ochratoxin A /kg b.w.) once daily. Citrinin concentrations were measured in plasma by an in-house validated HPLC method in week 6, week 8 (day 56) and the last day of the experiment (day 57). Mean plasma concentration varied between 84 (week 6) and 111 (day 57) ng/ml in the animals treated with citrinin only. Significantly lower concentrations (69 (week 6) and 49 (day 57) ng/ml) were measured in animals treated with both

citrinin and ochratoxin A. The authors concluded that citrinin is more rapidly eliminated than ochratoxin A. No further kinetic analyses were presented in this study.

7.1.2. Carry over into animal products

Only one study was identified on the potential carry over of citrinin from animal feeds into edible tissues and eggs. Laying hens received feed with a citrinin concentration of 100 µg/kg for 6 weeks. Citrinin residues of 10.4 ± 2.12[11] µg/kg egg yolk, 6.16 ± 1.22 µg/kg egg white, 10.3 ± 1.75 µg/kg white muscles and 9.84 ± 1.45 µg/kg red muscles were measured (Abdelhamid and Dorra, 1990). No citrinin residues were detected after a withdrawal period of 2 weeks.

In conclusion, these data show that citrinin residues may occur in edible tissues and eggs following oral exposure of animals with contaminated feed materials.

7.2. Toxicity in experimental animals

7.2.1. Acute toxicity

Acute subcutaneous (s.c.) administration of citrinin at doses of 50, 75 or 100 mg/kg b.w. was lethal for guinea pigs; hyperpnoea and dyspnoea being common signs of morbidity (Ambrose and DeEds, 1946). Pathology of decedents included renal tubule necrosis, acute myocarditis and necrosis of gastric mucosa. However, none of five animals given 25 mg/kg b.w. died.

The acute s.c. LD_{50} for citrinin in rabbits was ~ 20 mg/kg b.w. (Ambrose and DeEds, 1946), with notable overt responses of lacrymation and salivation, and mild histopathological changes in the kidneys. At higher s.c. doses (50-75 mg/kg b.w.) given for up to 14 days, citrinin caused marked renal lesions, confirming rabbits as sensitive to citrinin much like guinea pigs (Ambrose and DeEds, 1946). Also in the rabbit, a 2 % aqueous solution of citrinin caused conjunctival irritation when instilled into the conjunctival sac. Hanika et al. (1983) determined intraperitoneal (i.p.) and oral 72-hr LD_{50} values of 50 mg/kg b.w. and 134 mg/kg b.w., respectively. At a single oral dose of 120 mg/kg b.w., evidence of repair of renal damage (of tubular renal epithelium including necrosis) was seen when examined 7 days after dosing. Sub-lethal oral doses (33.5 or 77 mg/kg b.w.) given on seven consecutive days caused only mild renal changes together with regeneration of damaged tubular epithelium.

Phillips and Hayes (1978) studied alterations in liver and kidney in mice and observed pathological changes after a single i.p. dose of citrinin (35 mg/kg b.w.) confined to the liver including liposis in parenchymal and peripheral lobular zones, depletion of glycogen and increased parenchymal mitosis.

The LD_{50} of a single s.c. citrinin dose to rats was 67 mg/kg b.w. (Ambrose and DeEds, 1946). Sprague Dawley rats given once 50 mg/kg b.w. i.p. (Jordan et al., 1978b) showed within 3 hrs, glucosuria and haematuria and within 48 hrs indications of nephrosis. In another study, the same single dose given to the same rat strain induced only transient renal damage (Lockard et al., 1980). Similar dynamics of recovery from a large oral dose of the structurally-related mycotoxin ochratoxin A and illustrated by ^{1}H NMR urinary metabolomics (Mantle et al., 2011), shows the renal potential for recovery from, and tolerance of, these acute mycotoxicoses.

A single intravenous (i.v.) administration of citrinin (5 mg) to anaesthetised mongrel dogs caused a brief decrease in blood pressure, whereas 10 or 20 mg caused a severe decrease in blood pressure (Ambrose and DeEds, 1946).

The LD_{50} values quoted above and elsewhere in the literature (e.g. Carlton and Tuite, 1977), are summarised in Table 5, and appear to be in the same order of magnitude, in the mg/kg body weight range, with some difference in relation to the different routes of administration.

[11] Mean ± standard error of the mean.

Table 5: LD_{50} values of citrinin to experimental mammals as reported in the literature.

Animal	Administration route	LD_{50} (mg/kg b.w.)	Reference
Mouse	subcutaneous	35	Ambrose and DeEds, 1946
Mouse (male, organic vehicle)	subcutaneous	73	Jordan et al., 1977
Mouse	intraperitoneal	35	Ambrose and DeEds, 1946
Mouse (male, organic vehicle)	intraperitoneal	58	Jordan et al., 1977
Mouse (male, aqueous vehicle)	intraperitoneal	80	Jordan et al., 1977
Mouse (female, organic vehicle)	intraperitoneal	62	Jordan et al., 1977
Mouse (female, aqueous vehicle)	intraperitoneal	87	Jordan et al., 1977
Mouse (female)	intraperitoneal	89	Sansing et al., 1976
Mouse (male, organic vehicle)	oral	112	Jordan et al., 1977
Mouse (male, aqueous vehicle)	oral	105	Jordan et al., 1977
Rat	subcutaneous	67	Ambrose and DeEds, 1946
	intraperitoneal	67	Ambrose and DeEds, 1946
Guinea-pig	subcutaneous	37	Ambrose and DeEds, 1946
Rabbit	intravenous	19	Ambrose and DeEds, 1946
	intraperitoneal	50	Hanika et al., 1983
	oral	134	Hanika et al., 1983
Turkey poults	via crop	56	Mehdi et al., 1983
Ducklings	via crop	57	Mehdi et al., 1983

In summary, the acute lethal toxicity of citrinin ranged between 19 and 134 mg/kg b.w. depending on species and route of administration. Oral (intragastric) administration required a higher dose (> 100 mg/kg) for lethality in mouse and rabbit, indicating the limited oral bioavailability of citrinin is these animal species.

7.2.2. Short term repeat dose studies

Repeated daily i.p. injection (50 mg/kg b.w.) to five guinea pigs caused three deaths after the second dose and one each after subsequent doses (Carlton and Szczech, 1978). Histopathology in decedents was confined to congested kidneys, in which changes occurred through all parts of the nephrons, although glomeruli were unaffected.

In a study of Kitchen et al. (1977a, 1977b, 1977c) dogs were given two different doses of citrinin (5 mg/kg b.w. and 10 mg/kg b.w.) by i.p. injection for a period of 2 weeks. The highest dose was lethal with spontaneous mortality occurring after 7 to 11 days. All animals showed peritonitis following the i.p. injection of citrinin dissolved in ethanol. Histopathological investigations revealed tubular degeneration, karyorrhexis of nuclei and necrosis.

In guinea-pigs, a repeated dose study (described by the authors as for determining an LD_{50}), with oral administration of citrinin for 14 days to males and females, gave survival data from which a mean lethal dose of 43 mg/kg b.w. per day for both sexes was calculated (Thacker et al., 1977). The principal necropsy finding was swollen and discoloured kidneys.

Dietary exposure to citrinin at 250 and 500 mg/kg feed for two weeks was well tolerated by hamsters, with no clinical signs of toxicity, no gross lesions at necropsy and no histopathological changes were observed (Carlton and Szczech, 1978). If it is assumed that animals of 100 g weight consumed 10 g feed, the high dose of 500 mg/kg feed is equivalent to a 50 mg/kg b.w. dosage, similar to that having a marked toxic effect in guinea pigs.

Degenerative changes in renal proximal tubule epithelium were reported in rats given citrinin contaminated feed, but no dose was specified (Friis et al., 1969; Krogh et al., 1970). In contrast, no clinical or renal histopathological changes occurred in a rat given dietary citrinin for 5 days (~18 mg/kg b.w. per day) (Mantle and McHugh, 1993).

Experiments with three beagle dogs (Carlton et al., 1974) commenced with dry feed containing 50 % of rice moulded by *Penicillium citrinum* and containing 100 mg citrinin/kg. After 15 days of poor feed consumption and inappetance caused by citrinin, the rice content was reduced four-fold and the revised diet offered *ad libitum* for a further 17 days by which time signs of renal disease (e.g. dehydration and increased water consumption) were evident. Renal disease also occurred in three additional dogs given feed containing 25 % with the same moulded rice. In an additional study, Carlton and Szczech (1978) dosed orally beagle dogs (n = 3 per group) with pure citrinin (20 or 40 mg/kg b.w.), which resulted within 30 min in emesis and retching. Subsequent change to i.p. administration to by-pass the stomach still elicited severe responses (including polyuria) and by five days, all animals were dead or euthanized *in extremis*. The most sensitive indicator of citrinin toxicity in dogs given an oral dose of 5 mg citrinin/kg b.w. per day, was a marked increase in activity of lactate dehydrogenase in urine before clinical signs of renal disease occurred (Carlton et al., 1974).

In conclusion, all studies involving repeated administration of citrinin confirmed its nephrotoxicity. At the same time, these studies demonstrate significant species differences in the susceptibility to citrinin.

7.2.3. Subchronic toxicity

Recently a 90-day study with male Wistar rats (Lee et al., 2010), was conducted with the aim of establishing a safe concentration for dietary RMR as a food additive. In this study, citrinin was given in the form of RMR fermented with a mutant strain of *Monascus ruber* (NTU 505) that produces citrinin. This RMR contained different concentrations of citrinin (1, 2, 10, 20 and 200 mg/kg), and lovastatin (Table 6, group D to H). In addition, two groups of rats received unfermented rice to which citrinin was added in different concentrations (2 and 200 mg/kg) (Table 6, group B and C) and one group of rats received unfermented rice without citrinin (Table 6, group A). According to the authors, the animals consumed RMR/rice with the diet in a quantity of 100 mg/kg b.w. From the description of the experimental groups, the following information could be deduced (confirmed to the Panel by Pan T. through personal communication).

Table 6: Different concentrations of citrinin administered in a 90-day study with male Wistar rats (Lee et al., 2010).

Group	Citrinin concentration in RMR/rice[(a)] (mg/kg)	Calculated exposure dose for citrinin (µg/kg b.w. per day)	Lovastatin (mg/kg b.w.)[(b)]
A	0	0.0	-[(c)]
B	2	0.2	-[(c)]
C	200	20	-[(c)]
D	1	0.1	0.24
E	2	0.2	0.24
F	10	1.0	0.24
G	20	2.0	0.24
H	200	20	0.24

RMR: red mould rice
(a): this dosing regime was based on the assumption that an adult human individual will use a daily dose of 2 g RMR and corrected via the body surface coefficient to obtain the dose for rats.
(b): this dose refers to a standard dose in humans of 4.8 mg of monakolin A (the natural analogue of lovastatin) which is advised to be taken with RMR.
(c): unfermented rice that does not contain lovastatin.

Parameters tested were body weight gain, daily feed intake, organ weight and serum biochemistry as well as histopathology of livers and kidneys. Even at the highest dose tested, no toxicologically significant alterations were observed in this study, and hence the authors concluded that RMR containing citrinin at a level of 200 mg citrinin/kg RMR (equivalent to 20 µg citrinin/kg b.w. per day) was not nephrotoxic when tested in a 90-day study.

In conclusion, the results of this study suggest that a citrinin dose of 20 µg/kg b.w. per day can be considered as a NOAEL for rats. No other subchronic toxicity studies, which could be used to identify a NOAEL, are available in the open literature.

7.2.4. Nephrotoxicity

Arai and Hibino (1983) studied 6 weeks old male F344 rats in two groups: 50 rats were fed citrinin (99 % pure) blended (0.1 % w/w corresponding to 1000 mg/kg) with a commercial powdered chow and given ad libitum for 80 weeks and compared with 22 untreated rats which served as controls. Subsets of animals were killed and examined histopathologically and ultrastructurally after 32, 40, 60 and 80 weeks (see Table 7). Body weight in the treated rats declined throughout the experiment, which makes it difficult to estimate actual dosage. However, an initial feed intake of 20 g would have resulted in an exposure of approximately 70 mg citrinin/kg b.w. The treated group showed an increase in the ratio of kidney weight to body weight at each examination and an increase in the ratio of liver weight to body weight at 80 weeks (both statistically significant with $p < 0.05$), when at the same time the weight of the treated animals decreased, but not so for the controls. Large kidneys with fine granular surface were seen at each examination time. Histopathologically, the kidneys showed from the first examination marked dilatation of proximal convoluted tubules, colloid casts in the tubular lumina and interstitial fibrosis due to interstitial nephritis, as well as focal hyperplasia of renal tubules. Starting from week 40 all treated rats showed focal hyperplasia of the tubular epithelium and small adenomas. Massive benign clear cell adenomas occurred after week 60 in the kidneys. They were characterised ultrastructurally as having clear and dark cytoplasm with few to numerous organelles, and with dilatation of rough and smooth endoplasmic reticulum. No tumours were seen in kidney or other organs in the controls such that the difference in incidence of tubular dilatation, focal hyperplasia or adenomas in treated animals was statistically significant. Whether renal tumours were unilateral or bilateral was not recorded.

Table 7: Histopathological findings of kidney of rats treated with a 0.1 % citrinin diet (Arai and Hibino, 1983).

Group	Week of termination	Number of animals	Tubular dilatation	Focal hyperplasia	Adenoma
Citrinin (1g/kg feed)	32	13	13	13	0
	40	8	8	8	8
	60	17	17	17	17
	80	10	10	10	10
Sum		48	48	48	35
Control	32	7	0	0	0
	60	5	0	0	0
	80	10	0	0	0
Sum		22	0	0	0

In conclusion, only one controlled long term feeding study was identified, in rats with one dose group, with a high dietary exposure to citrinin (initially ~70 mg/kg b.w. per day). Kidney as the principal target organ showed progressive histopathological changes and high incidences of adenomas. This study was limited to a maximum period of 80 weeks, which is somewhat shorter than the normal duration of a rodent carcinogenicity study, i.e. at least 2 years (104 weeks). Hence, given the observed high incidence of adenomas it cannot be excluded that carcinomas would have occurred if exposure time had been increased to the full length of a carcinogenicity study.

7.2.5. Immunotoxicity

Most studies on the effect of citrinin on the immune system are rather old and in most cases studies were not focused on immunology. Carlton et al. (1974) found in a study on beagle dogs inconsistent changes in leukocyte counts which were probably more related to dehydration of citrinin treated

animals than to any direct effect of citrinin (20 and 40 mg/kg b.w. for 2 days administered in gelatine capsules and after that i.p. because of profound emetic effects). In a study on haematological parameters of citrinin treated mice (20 mg/kg b.w.; i.p. injections once a week for 6 weeks) a decrease in the total count of bone marrow cells (precursors of erythrocytes, leucocytes and megakaryocytes) was found (Gupta et al., 1983). In a LD_{50} study with hamsters, citrinin caused slight to mild necrosis of scattered individual lymphocytes in the spleen and the intestinal submucosa (Jordan et al., 1978a). Single oral doses of citrinin (50, 70 and 80 mg/kg b.w., respectively) which are similar or higher than the LD_{50} (56 mg/kg b.w.) caused in turkey poults necrosis and depletion of lymphocytes in the thymus and cloacal bursa (Mehdi et al., 1983). In thymus mild to marked necrosis of lymphocytes occurred mostly in the cortex, in contrast to the cloacal bursa where the necrosis and lymphoid depletion was more prominent in the medulla. The same distribution of lesions was observed in 7-day old ducklings (n = 10) treated orally with a single dose (30, 40 and 50 mg/kg b.w.) of citrinin and this study confirmed the cytotoxicity of citrinin to splenic lymphocytes *in vitro* (Mehdi et al., 1983).

Immunity was studied in citrinin-treated mice (0.12, 0.6 or 3.0 mg/kg b.w., i.p. every other day for 2 or for 4 weeks; n = 5 per group; Reddy et al., 1988). An increased uptake of ^{3}H-thymidine in DNA of splenic lymphocytes of animals given 0.6 mg/kg b.w. for two weeks or given 0.12 and 0.6 mg/kg for four weeks indicated their proliferation. A substudy on splenic lymphocyte cell culture from untreated animals supported this dose-response effect, being more prominent in the presence of lipopolysaccharide and pokeweed mitogen. Antibody production evaluated by measuring plaque formation after sensitization of splenic cells to sheep red blood cells (SRBC) was significantly higher in animals treated with citrinin at 0.12 and 0.6 mg/kg b.w., but not in animals treated with 3.0 mg/kg b.w. After two weeks of exposure the number of white blood cells was significantly decreased in animals given 0.6 and 3.0 mg/kg b.w. This reduction was mostly due to the reduction in lymphocyte counts, because the number of neutrophils and monocytes was not changed. Such decrease of peripheral leukocyte count was not seen in animals treated for four weeks. Taken together, these results indicate that citrinin mildly stimulates the immune system, but the immunotoxic effect is not consistent at all tested doses. This is in contrast to the more recent findings of Carvalho et al. (2005) on albino mice given a single citrinin dose (2.5 mg/kg b.w.) before, simultaneously, or after immunization with SRBC (3 repeated experiments with five animals per group). Decreased antibody titres were found in all citrinin-exposed animals. The exposure to citrinin before and after SRBC sensitization caused in both cases a decrease of 87.5 % in antibody titres, while when the application was simultaneous, the decrease was 75 %. The circulating complement was significantly reduced in animals given citrinin before and simultaneously with SRBC (87 % and 93.8 %, respectively), but when citrinin was given after SRBC stimulation, the complement levels remained unchanged as compared to that in animals given SRBC alone.

In human peripheral blood mononuclear cells (PMBC) incubated with citrinin a MTT test was performed to check cell viability and proliferation. T-cell function by the expression of cytokines was examined using ELISA, and intracellular cytokine staining and real-time polymerase chain reaction for interferon-γ (IFN-γ), and interleukin-4 (IL-4) was performed (Wichmann et al., 2002). Viability of PBMC was less affected by citrinin than cytokine release. The 50 % inhibitory (ID_{50}) dose of citrinin for IFN-γ was 8.3 µg/ml and for IL-4 21.6 µg/ml. The decreased production of IFN-γ without cytotoxicity, when PMBC cells were exposed to citrinin was confirmed by others (Tammer et al., 2007).

In conclusion, the studies of the immunotoxicity of citrinin are incomplete and often non-specific and do not allow a conclusive evaluation.

7.2.6. Developmental and reproductive toxicity

Limited data are available regarding the effects of citrinin following exposure of the embryo and foetus. Hood and co-workers described in 1976 that citrinin is an embryocidal and foetotoxic agent (Hood et al., 1976). In rats which received a single s.c. dose of 35 mg citrinin/kg b.w. on days 3 - 15 of gestation, no skeletal malformations of the foetuses were observed. However, enlarged kidneys,

internal hydrocephalus and cleft palates were found. As in this experiment 30-50 % of the pregnant dams died and the resorption rate of foetuses in the treated group was higher than in controls, it needs to be considered that maternal toxicity has influenced the outcome of this study. Kinetic investigations in pregnant rats provided no conclusive data about the percentage of citrinin that crosses the placenta (Reddy et al., 1988).

In a study conducted in a series of experiments, the effect of citrinin was investigated in pregnant Wistar rats and their offspring. Citrinin was administered in the feed at a concentration of 10 mg/kg feed (equivalent to approximately 1 mg/kg b.w.) during the gestation period (6 - 20 days post coitum). In the same experiments, endosulfan (1 mg /kg b.w.) and a combination of endosulfan (1 mg /kg b.w.) and citrinin (10 mg/kg feed) were tested. Investigations of the dams revealed mild maternal toxicity in the form of degenerative liver changes, multiple renal lesions and glomerular congestion when exposed to citrinin. In the intestines, a significant increase in the activity of global cells was noted along with mild to moderate, non-specific changes found during the histo-pathological investigations in other organs. Maternal weight gain and feed intake were reduced and various animals showed polyuria and polydypsia, a syndrome indicative of renal damage (Singh et al., 2006, 2007a).

In the same study, foetal resorption rate was increased and 6.8 % of the examined foetuses showed severe malformations, including internal hydrocephalus and notched and contracted kidneys (Singh et al., 2007b). About 10 % of all foetuses were retarded with incomplete ossification of the skull bones. Histological investigations of the foetal kidneys showed tubular degeneration, medullar tubular necrosis and interstitial fibrosation (Singh et al., 2008).

In conclusion, this study performed by Singh and co-workers and published in detail in four different scientific articles confirmed that citrinin induces teratogenicity in rodent species. It should be noted that the given dose induced maternal toxicity and the extent of offspring exposure could not be determined in this study. Moreover, data on the actual exposure remain inconsistent, as all dams showed reduced feed intake at variable levels. Hence, the deviation from the targeted dose (1 mg/kg b.w.) is considerable and contributes to the uncertainty of this study, which was conducted with only one dose/feed concentration.

The embryotoxic potential of citrinin was also investigated in the chick embryo model (range of citrinin tested 1 - 10 µg, injected subgerminally or intra-amniotically at different days of the egg incubation period) alone and together with ochratoxin A. The most pronounced alterations in the early embryonic development were observed following an exposure on day three, including embryonic death (up to 60 %), and embryos showed morphological alteration on their heads (exencephaly, micro-ophthalmia and cleft beak) (Vesela et al., 1983). These data are in line with earlier studies by Ciegler et al. (1977) describing also exencephaly, exopthalmia, crossed beaks and occasional crooked necks.

Recent *in vitro* studies with mouse embryonic cells confirmed the previous *in vivo* data as blastocysts treated with citrinin showed apoptosis and significant decreased implantation rates (Chan and Shiao, 2007). An increased rate of apoptosis was observed in mouse embryoblasts following *ex vivo* treatment with citrinin at a concentration of 30 µg/ml (Chan, 2007). In further *in vitro* experiments, Chan (2008) observed a significant reduction in the rate of oocyte maturation, fertilization and embryonic development.

Qingqing et al. (2010) investigated the effects of citrinin on the reproductive organs of male mice. Adult male Kunming mice (of 4 months of age) received an intraperitoneal injection of citrinin in four different doses (0, 0.0625, 0.625 and 6.25 mg/kg b.w., respectively), daily for 7 days. At the end of the exposure period reproductive organ relative weights, semen quality, serum testosterone concentrations and fertility of treated mice were assessed. Citrinin at all dosages significantly increased the relative organ weights of the testes, epididymis, seminal vesicle and preputial gland. Sperm counts were decreased and the number of abnormal spermatozoa was increased. Histological examination revealed an increased diameter of the testicular seminiferus tubule. Serum testosterone concentrations were decreased and a significantly lower pregnancy rate was observed when females were mated with the

citrinin-exposed males and no embryos occurred in the females mated with males given the highest dose of 6.25 mg/kg b.w.

In conclusion, *in vitro* and *in vivo* studies provided clear evidence for reproductive toxicity and, teratogenic and embryotoxic effects of citrinin. The doses tested in the *in vivo* experiments exerted, however, clear signs of maternal toxicity, including nephrotoxicity, indicating that these effects might be secondary to maternal toxicity. In male mice, a recent repeated dose study showed significant alterations in male reproductive organs and sperm quality at the lowest dose of 0.0625 mg/kg b.w. per day. It needs to be emphasized that citrinin was given in the latter study by intraperitoneal route.

7.2.7. Genotoxicity

Genotoxicity of citrinin has been tested in bacteria and mammalian cells *in vitro* and in mice *in vivo* (Table 8). In the bacterial tests with *Salmonella* Typhimurium strains TA1535, TA1538, TA98, TA100 and TA97 citrinin was not mutagenic either with or without S9-mix mediated metabolic activation (Wehner et al., 1978; Sabater-Vilar et al., 1999; Knasmüller et al., 2004). Citrinin, as well as ochratoxin A and patulin alone or in combinations did not induce mutations in *Salmonella* Typhimurium TA102, a strain specifically used for testing oxidative DNA damage (Würgler et al., 1991). A positive response was detected in *Salmonella* Typhimurim TA98 following a non-standard protocol, in which primary rat hepatocytes were used as metabolic activation system (Sabater-Vilar et al., 1999). Citrinin did not induce SOS responses in *Escherichia coli* PQ37 with or without S9 mediated metabolic activation (Malaveille et al., 1991; Sakai et al., 1992), and was negative in *Bacillus subtilis* rec assay (Ueno and Kubota, 1976). However, citrinin caused single-strand breaks in the chromosomal DNA of *Escherichia coli* and induced DNA repair synthesis in permeabilized *E.coli* cells (Martin et al., 1986).

In V79 hamster cells citrinin induced chromosomal aberrations following activation by rat and human liver microsomes, whereas it did not induce sister chromatid exchanges (SCEs) (Thust and Kneist, 1979). Citrinin also did not cause any increase in SCE frequency in Chinese hamster ovary cells or in human lymphocytes (Liu et al., 2003).

Pfeiffer et al. (1998) investigated the aneuploidogenic and clastogenic potential of citrinin. Citrinin inhibited microtubule polymerization under cell-free conditions in a concentration-dependent manner but did not bind covalently to microtubule proteins or other thiol compounds. In cultured Chinese hamster V79 cells, at concentrations that did not significantly affect cell viability (20 – 40 µM), citrinin caused mitotic arrest that was not time-dependent and reverted after the removal of the toxin. Micronuclei frequency was also significantly increased and micronuclei were all CREST-positive i.e. they contained whole chromosomes/chromatids. These observations indicate that citrinin induces aneuploidy, but not clastogenic effects. Citrinin was reported to be an equally potent inducer of micronuclei as ochratoxin A in the human-derived liver cell line HepG2, but, in contrast to ochratoxin A, it did not induce DNA single-strand breaks as measured by the comet assay (Knasmüller et al., 2004). In agreement with the study by Pfeiffer et al. (1998), micronuclei were predominantly centromere positive (78-82 %) indicating that they originate from induction of aneuploidy by citrinin. The induction of micronuclei by citrinin was more recently (Dönmez-Altuntas et al., 2007) confirmed in isolated peripheral human lymphocytes. Lymphocyte cultures were treated for 48 h with citrinin at doses ranging between 10 and 100 µM and a significant dose-dependent induction of micronuclei was observed.

When human peripheral blood mononuclear cells were exposed to citrinin, the percentage of cells with numerical chromosome changes was increased by 4.3-fold over that of vehicle-treated group (Chang et al., 2011). Results of this study suggested that disruption of microtubule organization by citrinin may contribute to the induction of numerical chromosome aberrations in human cells (see Section 7.3).

Liu et al. (2003) explored whether citrinin induces oxidative DNA damage. In human embryonic kidney cells (HEK293) exposure to citrinin did not induce DNA breaks and formamidopyrimidine-

DNA glycosylase (FPG) sensitive sites as measured by the comet assay thus excluding both direct DNA breaking activity as well as oxidative DNA damage induction. Citrinin also did not increase the mRNA level of human 8-hydroxyguanine DNA glycosylase 1 (hOGG1), while the expression of the heat shock protein 70 (HSP70) was elevated.

By using the ^{32}P postlabelling technique Pfohl-Leszkowicz et al. (2007) described three different DNA adducts in human kidney cells (HK2 cells) exposed to citrinin concentrations up to 50 µM. The chemical nature of these adducts was not elucidated. Formation of the same adducts was also reported in human tumour kidney tissues. As the nature of these adducts remains unknown, these findings cannot be further evaluated.

Citrinin (20 μg/ml), after 24 h exposure, induced the formation of foci of NIH/3T3 and C3H/10T1/2 cells colony formation in soft agar (Mehta and Kitabatake, 2002). These results show that citrinin has the ability to transform cells, and the authors concluded that citrinin has the potential to be carcinogenic.

Whether citrinin is genotoxic *in vivo* was addressed by a study conducted in mice after oral administration of citrinin (Jeswal, 1996). Both gross (hypoploidy, stickiness and clumping of chromosomes) as well as specific chromosome abnormalities were described in bone marrow cells after oral administration of citrinin (5 to 20 mg/kg) to young weanling mice for 8 weeks. The frequency of chromosomal abnormalities increased from 5.5 % found in controls up to 36 % in treated animals. Structural chromosomal changes observed were chromosome/chromatid breaks, gaps, acentric fragments, metacentric chromosomes and ring formations.

In conclusion, the available literature data indicate that citrinin is not mutagenic in conventional bacterial assays either with or without metabolic activation by S9 fraction from rat or human liver or rat kidney. Mutagenicity in the Ames test was only reported in one study when rat hepatocytes were used as activating system. In mammalian cells *in vitro,* citrinin did not induce DNA single-strand breaks, oxidative DNA damage or SCEs but induced micronuclei, aneuploidy and chromosomal aberrations. *In vivo* it induced chromosome abnormalities and hypodiploidy in the bone marrow of mice.

Table 8: Overview of genotoxicity studies.

Organism	Dosing	Exogenous activation	Endpoint	Mutagenic/ Genotoxic effect	Reference
***In vitro* studies**					
Salmonella Typhimurium	0.2 - 400 µg/plate	+ or - S9 fraction	his + revertants	-	Wehner et al., 1978
Salmonella Typhimurium	12 - 325 µg/plate	+ or - S9 fraction	his + revertants	-	Würgler et al.,1991
Salmonella Typhimurium	100 - 800 µg/plate	S9 Rat	his+ revertants	-	Sabater-Vilar et al., 1999
	0.25 - 50 µg/plate	Rat hepatocytes		+	
Salmonella Typhimurium	25 - 200 µg/plate	S9 from HepG2 cells	his+ revertants	-	Knasmüller et al., 2004
Salmonella Typhimurium	400 µg/plate	+ or - S9 fraction (rat)	his+ revertants	-	Kuczuk et al., 1978
Saccharomyces cerevisiae	50 and 100 µg/plate		mitotic recombination	-	
Salmonella Typhimurium	500 µg/plate	+ or - S9 fraction (rat)	his+ revertants	-	Ueno et al., 1978
Escherichia coli PQ37	0.1 - 24 µg/ml	+ or - S9 fraction (rat)	SOS response	-	Sakai et al., 1992
Escherichia coli PQ37	100 - 4000 µM	No	SOS response	-	Malaveille et al., 1991
Escherichia coli	100 µg/ml	No	DNA breaks	+	Martin et al., 1986
Bacillus subtilis M45	100 µg/disc	No	Recombination repair	-	Ueno and Kubota, 1976
HEK cells	15 µM	No	DNA breaks, oxidative DNA damage	-	Liu et al., 2003
CHO, human lymphocytes	5 - 15 µM	No	SCE		Liu et al., 2003
HepG2 cells	> 2.5 µg/ml	No	Micronuclei	+	Knasmüller et al., 2004
			DNA breaks	-	
V79 E Chinese hamster cells	250 - 1000 µM	human and rat liver S9 rat kidney S9	Chromosome abnormalities, hypodiploidy	+	Thust and Kneist, 1979
V79 Chinese hamster cell	40 µM	no	Aneuploidy, mitotic arrest and micronuclei in cultured cells	+	Pfeiffer et al., 1998
NIH/3T3 and C3H/10T1/2 cells	20 µg/ml	No	cell transformation	+	Mehta and Kitabatake, 2002
Human lymphocytes	60 - 100 µM	No	Micronuclei	+	Dönmez-Altuntas et al., 2007
Human peripheral blood mononuclear cells	50 µM	No	Numerical chromosomal aberrations	+	Chang et al., 2011
***In vivo* study**					
Young weanling mice	5 to 20 mg/kg b.w., 8 weeks	-	Chromosome abnormalities, hypodiploidy in bone marrow	+	Jeswal, 1996

CHO: Chinese hamster ovary; SCE: sister chromatic exchange.

7.2.8. Carcinogenicity

IARC (1986) concluded that there was limited evidence for the carcinogenicity of citrinin to experimental animals and that no evaluation could be made of the carcinogenicity of citrinin to humans. Citrinin is classified in group 3 (not classifiable as to its carcinogenicity to humans).

In an 80-week chronic toxicity study (see Section 7.2.4), all treated rats showed, starting from week 40, focal hyperplasia of the tubular epithelium and small renal adenomas but no carcinomas or related urinary tract tumours were observed (Arai and Hibino, 1983). The duration of this study was limited to 80 weeks, and hence it cannot be excluded that carcinomas would have occurred following a longer exposure and/or observation period.

Hatanaka et al. (1982) tested the carcinogenicity of citrinin in a model fish species (*Oryzias latipes*) which was exposed to citrinin for 24 weeks. No evidence for carcinogenicity was found.

In an earlier study, Shinohara et al. (1976) considered whether dietary citrinin could act as a tumour promoter in male Sprague-Dawley rats. A pilot experiment with an initial oral intake of ~70 mg/kg b.w. per day did not result in renal tumours. In the final experiment, the animals were given dietary N-nitrosodimethylamine (NDMA) during 2 weeks at an initial intake of ~70 mg/kg b.w. per day and then given the citrinin regimen for 20 weeks either at an oral dose of ~70 mg/kg b.w. per day or at ~ 25 mg/kg b.w. per day. Extensive multiple *in situ* renal tumourigenesis occurred bilaterally or unilaterally in 13 out of 15 animals in the low and 18 out of 19 in the high dose group, and most tumours were designated as renal cell (adenoma) type. In contrast, less tumourigenesis occurred in a group given only the NDMA treatment (tumours in 8 out of 14 animals) and most tumours were designated as embryonal cell type. The conclusion was that citrinin changed the type, and increased the incidence of renal tumours after exposure to NDMA.

In conclusion, these experiments show that citrinin induced renal adenomas in rodents at a very high frequency in an 80 weeks chronic toxicity study. Although no renal carcinomas or other tumours were reported in this study, no conclusion can be drawn regarding the potential carcinogenicity of citrinin because of the lack of appropriate long-term (2-year) studies.

7.2.9. Interactions between citrinin and ochratoxin A, and citrinin and patulin

Humans and animals are frequently exposed to several mycotoxins, albeit at low concentrations of individual mycotoxins. The relevance of interactions between citrinin and other mycotoxins, in particular ochratoxin A, for assessing the toxicity of citrinin has been discussed repeatedly. In referring to the terms of reference, the CONTAM Panel examined available publications addressing interactions of citrinin with patulin, aflatoxins and ochratoxin A, particularly on the subject of synergism. Studies on interactions of citrinin with penicillic acid, cyclopiazonic acid and roquefortine are also available but not discussed in this opinion.

One recent experiment with chickens involved dietary exposure to aflatoxin B_1 (0.5 mg/kg feed) and citrinin (150 mg/kg feed), alone or combined (Ahamad et al., 2006). Citrinin suppressed feed intake and caused renal pathology while aflatoxin characteristically affected liver, but there was no evidence for synergism when both toxins were given concomitantly.

Evidence for an increased toxicity in cases of co-exposure to citrinin and ochratoxin A was provided by an acute toxicity study in 120 mice (Sansing et al., 1976). In this study, only lethality was measured, within three days after an intraperitoneal administration. The extreme nature of the test, the idiosyncrasy of individual responses in succumbing to the toxic insults, and the relatively small group sizes (n = 6 per group with a total of 20 groups with different dosages of citrinin and ochratoxin A tested) gave results which did not allow any estimation of a dose-response. Nevertheless, lethality in all combinations of citrinin and ochratoxin A was greater than was attributable simply to an additive response to the toxins alone.

Kanisawa (1984) reported no renal histopathological changes in male mice given dietary citrinin (estimated as ~ 13 or 26 mg/kg b.w. per day) life long (70 weeks). In contrast, ochratoxin A (at ~ 3 mg/kg b.w. per day) generally caused renal histopathological change, and induced renal tumours in 30 % of the animals. Regimens combining the dietary ochratoxin A dose with citrinin at either the 13 mg/kg b.w. per day or the 26 mg/kg b.w. per day intake, gave surprisingly either no renal tumours or a 56 % incidence, respectively. The difference in renal tumour incidence between the latter combination group and the ochratoxin A alone group was statistically not significant. Therefore, in contrast to the conclusions made by the authors, the presence of an interaction is not substantiated by statistical analysis and the CONTAM Panel noted that a complete suppression of ochratoxin A renal tumourigenesis at 13 mg citrinin/kg b.w. per day and an incidence of 56 % at 26 mg/kg b.w. per day does not provide experimental evidence for a synergistic influence of citrinin on experimental ochratoxin A tumourigenicity, even at the high intakes.

A previous review of the literature on ochratoxin A prepared by Pohland et al. (1992) for the International Union of Pure and Applied Chemists (IUPAC) investigated the interaction of ochratoxin A with other mycotoxins and concluded that synergism could occur but with several examples of only an additive relationship or even of antagonism depended on the biological system used. Shortly after, Braunberg et al. (1994) used pig kidney cortex cubes, exposed *in vitro* for an hour to citrinin and ochratoxin A alone or in combination in the range $1 - 10^{-3}$ mM. The tissues were then re-incubated to measure transport of tetraethylammonium or paraminohippurate ions, as well as synthesis of proteins from ^{3}H-leucine. Inconsistent results were obtained for the effects in all three parameters, which did not support the hypothesis of synergism in response to combinations of citrinin with ochratoxin A.

Bernhoft et al. (2004) studied pair combinations at five concentrations of six mycotoxins, including citrinin, in a mitogen-induced lymphocyte proliferation assay. Some combinations were additive in growth inhibition, some were less-than-additive and others were independent. The synergistic multiplicity of response relative to theoretical additive response in the study conducted by Sansing et al. (1976) of citrinin with ochratoxin A was of the order of 2-fold, at large i.p. doses of citrinin around the LD_{50} value of 89 mg/kg b.w. Synergism was of similar magnitude (~ 3-fold) in the *in vitro* study of Bernhoft et al. (2004) at the higher doses (showing synergism at combinations of 2 mg/L and 0.36 mg/L, for citrinin and ochratoxin A respectively, and at greater concentrations). No such synergistic response of cells was observed *in vitro* at a citrinin concentration of ~ 1 mg/L. Notably, citrinin was not synergistic with patulin in this *in vitro* study.

Heussner et al. (2006) reported an *in vitro* synergistic effect of citrinin with ochratoxin A in cultured cells exposed to the toxins at ~ 120 µM and 12 µM, respectively. The citrinin concentration in this experiment was equivalent to 30 mg/L and a synergistic effect at this high concentration is consistent with the findings of Bernhoft et al. (2004). A more recent study of individual and combined exposure of mice to citrinin and ochratoxin A, in doses of 2, 4 or 8 % of their LD_{50} value, measured chromosomal aberrations in bone marrow cells of mice 24 h after intraperitoneal injection (Bouslimi et al., 2008). They showed that combined toxic responses at the lowest dose equated with the sum of individual dose responses. At the higher doses the combined toxin response progressively failed even to achieve an additive value of the responses to a toxin alone. Authors claimed a clear synergistic relationship between citrinin and ochratoxin A in the abstract, which is not supported by the experimental findings. Therefore, their claim and possible relevance of synergism for explaining the molecular basis of mycotoxic renal disease and tumourigenesis is unsustainable. Thus, at a dosage of at least 10-fold less than that used by Sansing et al. (1976), an assumption of synergism cannot be justified.

Additional references on the investigation of synergism between citrinin and ochratoxin A have been cited in a recent review (Grenier and Oswald, 2011) and are evaluated here. Manning et al. (1985) gave broiler chicks citrinin and/or ochratoxin A for 3 weeks at 300 mg/kg feed or 3 mg/kg feed, respectively. Both toxins alone caused growth depression, and haematolological and histopathological changes. However, not only was there no evidence of additive or synergistic effects in combination, the combination even ameliorated aspects of the toxicoses. The same authors (Brown et al. 1986) gave

additional detail on renal ultrastructure in the broiler chick experiment. Kumar et al. (2008) made an exclusively-immunological study in rabbits given 15 mg citrinin/kg feed or 0.75 mg ochratoxin A/kg feed, or in combination, for 60 days. Whereas citrinin alone evoked little effect on cell-mediated immunity, in combination with ochratoxin A, it appeared to add to the well-known immuno-supressive characteristic of ochratoxin A. The absence of any comment on the animal's health through the experiment indicates absence of adverse clinical effects of citrinin at 15 mg/kg feed.

Kitchen et al. (1977b), in their experiments with dogs given ochratoxin A alone or with high intraperitoneal doses of citrinin (5 or 10 mg/kg b.w.) daily for up to 14 days, specifically concluded that 'whether or not the increased mortality caused by the administration of combined toxins represents true synergism or only additive toxicity was not established'. This contrasts with the assertion in Grenier and Oswald (2011) that synergistic action was observed. Vesela et al. (1983), in their studies on chick embryos found additive but not synergistic effects of combining citrinin with ochratoxin A. However, Siraj et al. (1981) studied neonatal rats given a single oral dose of ochratoxin A and or citrinin (the latter at the high dose of 25 mg/kg b.w.) and reported synergistic responses of some renal enzymes. Mayura et al. (1984) studied pregnant rats given ochratoxin A and/or citrinin (the latter at 30 mg/kg) alone or combined at stages during gestation. There was considerable maternal mortality but combined toxins enhanced prenatal toxicity by comparison with toxins alone. It is notable, however, that the foregoing experimental studies were with extreme exposure of animals at very vulnerable stages in life. In a recent *in vitro* study, the role of calcium in apoptosis and aberrant chromatin forms in porcine kidney PK15 cells investigated also the interaction of ochratoxin A and citrinin showing at most an additive effect (Šegvić et al., 2012; see Section 7.3).

The available evidence indicates that citrinin at low doses does not exacerbate the toxic effects of other mycotoxins. The CONTAM Panel concluded that the combined effect of citrinin and ochratoxin A is at most additive.

7.3. Modes of action

Studies on the mechanism of citrinin toxicity were performed exclusively *in vitro*. Inhibition of RNA (predominantly rRNA) and DNA synthesis by citrinin has been reported in different mammalian cell lines including kidney cells (Wasternack and Weisser, 1992) (Yoneyama and Sharma, 1987).

The induction of oxidative stress has also been proposed to account for citrinin toxicity. The induction of lipid peroxidation as measured by increased malondialdehyde (MDA) levels in Vero kidney cells (Bouslimi et al., 2008), increased expression of the heat shock protein HSP70 in Vero cells (Bouslimi et al., 2008) and human embryonic kidney cells HEK293 (Liu et al., 2003), significant depletion of the antioxidant glutathione (GSH) at sub-toxic doses in alveolar epithelial cells (Johannssen et al., 2007) as well as microarray studies in yeast showing induction of oxidative stress-related genes (Iwahashi et al., 2007) are in favour of a role of oxidative damage in citrinin-induced toxicity. However, no evidence of oxidative DNA damage induction, as measured by single cell gel electrophoresis (SCGE), nor of activation of oxidative DNA damage repair genes such as the 8-hydroxyguanine DNA glycosylase 1 (hOGG1) were reported in citrinin-exposed cell cultures (Liu et al., 2003).

Using suspensions of rat renal proximal tubules the cellular events associated with renal cell death by citrinin were investigated (Aleo et al., 1991). Citrinin had multiple effects on mitochondrial function, impairing mitochondrial respiration with subsequent loss in cellular adenosine triphosphate (ATP) levels. Iron-mediated oxidative stress seems not to be a factor in citrinin induced effects on mitochondria and cell death as suggested by lack of protection by these effects in the presence of the chelating agent deferoxamine. Whether alteration of mitochondrial function is a primary or contributing mechanism to cell death by citrinin remains to be clarified. Alteration by citrinin of mitochondria with consequent swelling and cell death was later confirmed in baby hamster kidney cells (Chagas et al., 1994). Ribeiro et al. (1997) studied the effects of citrinin in the maintenance of the homeostasis of the reactive oxygen species (ROS) in rat liver cells. Citrinin was shown to inhibit some enzymatic antioxidant activities and to induce oxidative stress by increasing the formation of

superoxide anion in the mitochondrial respiratory chain. Citrinin seems also to affect the mitochondrial calcium transit as suggested by a study performed in porcine kidney PK15 cells incubated separately with citrinin (30 and 50 µM) and ochratoxin A (6 and 10 µM) or with their combinations (Šegvić et al., 2012). Simultaneous ochratoxin A and citrinin treatment had an additive cytotoxic effect but the effect on cytosolic calcium level was less than additive. When BAPTA-AM (Ca^{2+} chelator) was applied, it significantly reduced citrinin and ochratoxin A + citrinin induced apoptosis, thus showing the importance of Ca homeostasis in citrinin toxicity.

In a study on mouse blastocysts exposed to citrinin (15 and 30 µM) for 24 hours the cell viability was significantly decreased via citrinin-induced apoptosis (Chan and Shiao, 2007). In embryonic stem cells (ESC-B5) it was shown that citrinin triggers apoptosis via ROS generation, increased Bax/Bcl-2 ratio, loss of mitochondrial membrane potential, induction of cytochrome c release and inactivation of caspase 3 (Chan, 2007). In this model citrinin caused cell death by inactivation of the HSP90/multichaperone complex and subsequent inhibition of the Ras/ERK survival signalling. In a human osteoblast cell line it was shown that citrinin induces apoptosis via activation of c-Jun N-terminal kinase (JNK), loss of mitochondrial membrane potential, and caspase-3 and p21-activated protein kinase 2 (PAK2) activation (Huang et al., 2009). In another study in human embryonic kidney (HEK293) and HeLa cells citrinin was shown to positively regulate ERK1/2 and JNK pathways as well as their downstream effectors; activated mitogen-activated protein kinases pathways were also involved in citrinin induced apoptosis (Chang et al., 2009). In human promyelocitic leukemia cells (HL-60) citrinin-induced apoptosis was characterized by the activation of caspase-3, -6, -7 and -9, but not caspase-8 (Yu et al., 2006). The use of antioxidants did not suppress the apoptotic activity of caspase-3 suggesting that oxidative stress is not involved in the apoptotic process.

Liu et al. (2010) studied the immunomodulatory role of citrinin in nitric oxide (NO) production in glomerular mesangial MES-13 cells. Citrinin exerted an inhibitory action on lipopolysaccharide (LPS)/IFN-γ-triggered inducible nitric oxide synthase gene expression through the suppression of the transcriptor-1α signalling pathway. In turn, the stimulation of MES-13 cells with LPS/IFN-γ enhanced interferon response factor-1 mRNA expression while the co-supplementation with 25 and 40 µM citrinin reversed this effect. This study also shows that the presence of citrinin prevented the nuclear translocation of nuclear factor κB and phosphorylation of inhibitory factor –κB. These data indicate that the nephrotoxicity of citrinin may impair the host immune response by interfering with mesangial cell functions, such as NO release, one of the most important antimicrobial effectors during bacterial infection.

Exposure to citrinin of HEK293 cells (Chang et al., 2011) resulted in G2/M arrest of cell cycle, increased mitotic index, suppression of the microtubule assembly during interphase, and in spindle formation during mitosis suggesting that the cell cycle arrest induced by citrinin should primarily occur during the M phase. Citrinin was shown to inhibit tubulin polymerization in a cell-free system as well as in the cells. Increased expression levels of the DNA damage-inducible proteins p53 and p21 were also reported.

From the data available on mammalian cells in culture, the CONTAM Panel concluded that citrinin toxicity is exerted via multiple pathways such as DNA and RNA synthesis inhibition, inhibition of microtubule assembly and of tubulin polymerization, alteration of mitochondrial functionality with consequent increase in ROS production, inactivation of the HSP90 multichaperone complex and activation of the signal transduction pathway and the caspase-cascade system that result in apoptotic cell death.

7.4. Adverse effects in livestock and pet animals

7.4.1. Pigs

A case study by Friis et al. (1969) reported the results from pigs (30 kg b.w., n = 2 per dose group) administered citrinin in feed at 20, 40 or 100 mg/kg b.w. per day. The duration of exposure is unclear,

but is interpreted to be different for each pig ranging from 1 to 34 days. At the highest dose level, the pigs died in a coma with renal insufficiency and a decrease in appetite was observed in all animals. At 20 and 40 mg/kg b.w. per day growth depression, loss of weight and glucosuria were observed. The data are poorly reported and as such were not used for risk assessment.

Sándor et al. (1991) described a subacute toxicity study with ochratoxin A and citrinin in swine. Despite the limitations of the study in which only three animals were treated with citrinin over a period of 8 weeks, the data suggest that pigs are able to tolerate citrinin concentrations in feed resulting in doses of up to 0.02 mg/kg b.w. per day for a longer period without any signs of toxicity, as no alterations could be observed in the final haematological and histopathological investigations (enzymes, metabolites, total protein, haematological measures and urinary variables). Blood potassium increased after day 3 but no further subsequent increase was observed (reflecting transient renal dysfunction) and as was an initial decrease in beta-globulin. This study suggests a NOAEL of 20 µg/kg b.w per day for pigs.

Szczech et al. (1974) reported increased concentrations of glutamic oxalacetic transaminase, isocitric dehydrogenase and lactic dehydrogenase in serum and urine before clinical signs of renal disease were evident in pigs given oral doses of 1.0 and 20.0 mg citrinin/kg b.w. per day. The activities of the enzymes were elevated in urine, whilst no other signs of renal disease were observable, and there was no alteration in blood urea nitrogen. An early elevation in these serum activities is acknowledged to be a sensitive indicator of renal damage.

In conclusion, only few studies on adverse effects of citrinin in pigs could be identified. At present, no effect has been reported from pigs given 20 µg/kg b.w. per day (Sándor et al., 1991). The CONTAM Panel considered this intake value as a NOAEL, which is consistent with the results from a subchronic study in rodents (Lee et al., 2010), as described in Section 7.2.3.

7.4.2. Poultry

Toxic effects were not described in broiler chicks fed a diet containing 65 mg citrinin/kg feed (Carlton, 1980).

In a study administering 0, 33, 65, 130 or 260 mg citrinin/kg feed to broiler chicks (n = 5 per group of Cobb x Cobb colour-sexed male and female chicks) from one day old for 4-6 weeks, diarrhoea was observed at the two highest concentrations. At necropsy these chicks had haemorrhages in the jejunum as well as enlarged livers and kidneys. Chicks fed lower dose levels appeared normal macroscopically. All dietary levels resulted in lymphocyte and eosinophil infiltrations of the liver, kidneys and pancreas. The authors interpreted their qualitative observations of anaplastic areas of the kidney and pancreas, observed at the highest concentration of 260 mg/kg citrinin as of being suggestive that citrinin may be a carcinogen in chickens (Roberts and Mora, 1978).

Citrinin was fed to broiler chickens at concentrations of 300 mg citrinin/kg feed from one day of age until 3 weeks, and the birds were sacrificed on day 21. Compared to controls, birds fed citrinin had a significantly ($p \leq 0.05$) lower body weight on days 14 and 21 and an increased water consumption on days 7, 14 and 21. At the last day of the study, serum protein, albumin and globulin were significantly higher in the birds fed citrinin than in controls (Manning et al., 1985). Post-mortem investigations of these animals revealed mild renal structure changes, associated with proximal tubular intra-nuclear membrane-bound inclusions, misshaped mitochondria, as well as an increase in size and number of peroxisomes and secondary lysosomes. Birds fed the citrinin containing diet only to day 7, showed similar but milder changes (Brown et al., 1986).

Citrinin fed to mature laying hens at concentrations of 0, 50 or 250 mg/kg diet for three weeks had no effect on body weight, feed consumption, egg production, egg weight, or quality of eggshell. Moderate diarrhoea, which subsided once the birds returned to their normal diet, was observed after approximately three weeks at the highest dose level. Diet containing 0, 62.5, 125, 250 or 500 mg citrinin/kg feed given to broiler chicks from hatching to three weeks, resulted in a statistically

significant decrease in body weight at the highest dose level. All dose levels resulted in enlarged kidneys, and a slight dose related increase in liver weight (no histopathological examination was undertaken). The 250 and 500 mg/kg feed in the second study resulted in a dose related increase in water consumption, accompanied by acute diarrhoea (Ames et al., 1976).

Glahn and Wideman (1987) evaluated the dose/time-response effects of citrinin given at two concentrations of 200 and 400 mg/kg in two experiments (unilateral renal portal infusion for comparing a non-infusion with an infusion period or with systemic i.v. infusion together with parathyroid hormone) in 12 and respectively 4 immature Single Comb White Leghorns (about 1kg b.w.). Controls were treated with an ethanol solution only. Examining urethral urine after a maximum of 90 min, showed effects in the urine flow, free water clearance, fractional sodium excretion and urine osmolality but no effects on glomerular filtration rates, fractional potassium excretion or fractionated inorganic phosphate excretion. In a follow up study (Glahn et al., 1989) ten week old Leghorn pullets (n = 5 per group) were examined for renal function 10 days after administering 6 mg citrinin/kg b.w. Although citrinin induced effects on renal function (diuresis evident by higher urine flow rates, flow/glomerular filtration rate values and decreased osmolality), the duration of the effects was short.

Abdelhamid and Dorra (1990) examined the effects of a dietary concentration of 100 µg citrinin/kg feed in a six weeks feeding study, followed by two weeks recovery of 13 months old laying hens (Egyptian breed Mamourah). Citrinin-fed hens (three hens were checked after 6 weeks and another three after eight weeks) had enlarged spleen and enlarged reproductive organs. Investigating a large number of parameters (relative organ weights, blood parameters, chemical and physical characteristics in carcass muscles, liver characteristics, and bone minerals) the observed alterations in the citrinin group compared to controls were confined to changes in the relative organ weight of the adrenal glands, and compositional changes (fat/protein) in the liver, red and white muscle fibres. No renal lesions were visible during post mortem investigations. Residues of citrinin were found in eggs and muscles ranging from 6.2 to 10.6 ppb on fresh weight basis. This low concentration suggests a specific sensitivity of chickens towards citrinin, but has not been reported in other studies.

Mehdi et al. (1983) reported LD_{50} values of 56 mg/kg b.w. and 57 mg/kg b.w. for turkey poults and ducklings, respectively. Citrinin fed via the diet to ducklings (from one day old for 15 days) at 100, 250 and 500 mg/kg feed was observed to be nephrotoxic at 250 and 500 mg/kg feed, with the effects noted to be more severe in the highest dose group. Decreased weight gain and feed consumption was reported at both dose levels, and at autopsy the kidneys were observed to be 'swollen, pale and friable' with the tubular epithelial cells noted to be necrotic (Mehdi et al., 1984).

In summary, only a few studies on adverse effects of citrinin in poultry are available. Moderate effects have been reported from broiler chicks given 33 mg citrinin/kg feed (Roberts and Mora, 1978), which is equivalent to an intake level of 1.7 mg/kg b.w. per day assuming a body weight of 2 kg and a feed intake of 0.106 kg per day (equivalent to 0.12 kg dry matter per day). Another study (Abdelhamid and Dorra, 1990) reported effects in laying hens when administering 125 g feed per day containing 0.1 µg citrinin/kg feed to hens of 2 kg b.w., which is equivalent to an intake level of 6.25 µg/kg b.w. per day. Other studies showed effects at higher doses only. In conclusion, effects in poultry varied widely in type and severity depending on species, age of the birds and the design of the studies. The information available on the quality of feed and the feeding of the birds in these studies, and the data on animal weight at start and on weight during the study was limited such that a reliable intake level in terms of body weight per day could not be assigned to the different concentration levels tested in these experiments. Therefore, the CONTAM Panel considered the available data on poultry not suitable for risk characterisation and no NOAEL/lowast-observed-advers-effect level (LOAEL) for poultry could be identified.

7.4.3. Rabbits

In 16 young growing New Zealand White rabbits 6-8 weeks old (experimental and control groups of n = 4) fed citrinin contaminated feed at one concentration (15 mg/kg feed) for 60 days, general signs of toxicosis (anorexia, dullness, lethargy, loose faeces, polydypsia and dehydration) were noticed (Kumar et al., 2007). Investigation of utrastructural lesions in kidney of citrinin-treated animals focused on proximal convoluted tubules (PCT) and interstitial cells, while epithelial cells of distal convoluted tubules had almost normal appearance. In epithelial cells of the PCT citrinin caused loss of nucleoli, depletion of cytoplasmic organelles, peripheral condensation of pleomorphic mitochondria and cytoplasmic vacuolation. Degenerative and necrotic changes were mild to moderate. The basement membrane of PCT epithelial cells and glomerula were unaffected. Mitochondrial swelling and misshapen appearance are consistent with the theory that the lesions of this organelle are crucial in the mechanism of citrinin toxicity. Based on that qualitative assessment of ultrastructural characteristics of renal lesions of citrinin alone, compared with the groups fed ochratoxin A (0.75 mg ochratoxin A/kg feed or 0.75 mg ochratoxin A/kg feed + 0.15 mg citrinin/kg feed, respectively) an additive interaction in rabbits was suggested.

The same group of researchers (Kumar et al., 2008) reported on humoral and cellular immune response in a 60 days fattening study on 32 New Zealand rabbits (n = 8 per group) given the same dose as in the previous study administered with a standard basal diet plus fresh green fodder (200 g/animal). The citrinin treated animals showed a statistically significant decline in mean humoral immune response in antibody titres to SRBCs (mean of citrinin group of 3.75 (s.e. = 0.48) versus a control mean of 5.50 (s.e. = 0.29)). The humoral titre values, which were reduced to half of those of the control, were interpreted to constitute a relevant animal health effect e.g. when infections occur in the populations. The treated rabbits failed to show any significant changes in cell mediated immune response following the lymphocyte transformation assay and the delayed type hypersensitivity test.

No other repeated-dose studies on effects of citrinin in rabbits were identified. As both studies reported by Kumar (Kumar et al., 2007, 2008) do not provide information on body weights or body weight gain during the 60 days of treatment and feed consumption, no intake value could be calculated for this single dose group and no NOAEL/LOAEL for rabbits could therefore be identified.

7.4.4. Dogs

There are a few case reports of citrinin consumption in dogs, and those reporting dose and duration are included below.

In an early study, dogs were given citrinin dissolved in ethanol by intraperitoneal injection for a period of 2 weeks. Animals dosed with 10 mg/kg showed spontaneous mortality after 7 – 11 days. Animals that had been dosed with 5 mg/kg b.w. i.p. survived the entire study, but severe proximal tubule damage was observed during the post-mortem examination. (Kitchen et al., 1977a, b, c)

A case report describes five dogs having a common history of consumption of a dry dog food for over one month, which upon analysis was shown to contain citrinin at 8.3 µg/kg feed (and ochratoxin A at 372.8 µg/kg feed). The dogs were diagnosed with renal failure. Upon referral, they showed anorexia, emaciation, vomiting and polydipsia/polyuria and consumption of the feed was reported to be 'over one month'. The dogs had high blood urea nitrogen and creatinine. Two severely affected dogs died, while the others recovered gradually. Renal atrophy, congestion of the glomerula capillary, and diffuse degeneration, necrosis, dystrophic calcification and regeneration of the tubular epithelium were seen (Ahn et al., 2007).

A further case report of one dog consuming feed containing 150 µg citrinin/kg feed (duration not specified) presented with a severe scrotal dermatitis which did not respond to antibiotic and corticosteroid therapy. The dog made a rapid clinical recovery in response to glucocorticoid therapy. Histopathology indicated that the lesion was necrolytic and indicative of a hepatocutaneous syndrome. Increased levels of alanine aminotransferase, alkaline phosphatase, gamma-glutamyl transferase were

reported in plasma, which could be due to administration of corticosteroids, but were markedly higher than those induced experimentally by larger doses of glucocorticoids over longer dose periods (Little et al., 1991).

The CONTAM Panel concluded that the available data were too limited to allow a reliable risk characterisation for dogs.

7.4.5. Other animal species

Experimental data regarding systemic toxic effects in ruminants are not available. It is assumed that citrinin is highly degraded and metabolised through the microbial activity in the forestomachs of ruminants. However, an impairment of the rumen flora due to the antibacterial effect of citrinin cannot be excluded.

No data from other animals (e.g. fish) or companion animals other than dogs could be identified.

7.5. Observations in humans

A report on an acute human response to micro-crystalline citrinin from 30 L of stationary liquid fermentation broth dates back to 1931 and states that 'the very light yellow powder on inhalation gives rise to violent fits of sneezing' (Hetherington and Raistrick, 1941).

Ambrose and DeEds, (1946) reported an accidental inhalation of citrinin dust where mucosal irritation and nasal discharge was observed.

7.6. Critical effects and possibilities for derivation of a health based guidance value

The CONTAM Panel investigated the database for the availability of experimental data amenable for a dose-response analysis. Comparing the toxicological endpoints of nephrotoxicity immunotoxicity, hepatotoxicity, developmental and reproductive toxicity and carcinogenicity, the kidney was the principal target organ in all relevant studies analysed.

The study of Arai and Hibino (1983) investigated male F344 rats fed 1 000 mg citrinin/kg in the diet for up to 80 weeks (see Section 7.2.4). Tubular dilatation, focal hyperplasia, and adenoma occurred in the kidney in 100 % of the treated animals but in none of the controls. It was estimated that the given concentration of citrinin was equivalent to a dose of approximately 70 mg/kg b.w. per day. As only a single dose was tested, data were not used for a dose-response analysis.

The study of Lee et al. (2010) described a 90-day toxicity study conducted with male Wistar rats (see Section 7.2.3). No toxicological effects were observed at the two doses of 0.2 and 20 µg/kg b.w. per day tested. These data were used to identify a NOAEL of 20 µg/kg b.w. per day for nephrotoxicity and are supported by a 8-week study in pigs (Sándor et al., 1991) in which also a NOAEL of 20 µg/kg b.w. per day was identified (see Section 7.4.1). The CONTAM Panel noted that since no higher doses were used in the study of Lee et al. (2010), it does not provide information on effect levels and as such, no LOAEL could be derived.

In conclusion, these experiments show that citrinin induced nephrotoxicity including renal adenomas in rodents at a very high frequency in an 80-week chronic toxicity study. Although no renal carcinomas or other tumours were reported in this study, no conclusion can be drawn regarding the potential carcinogenicity of citrinin because of the lack of appropriate long-term studies.

Given the available data on genotoxicity, and the limitations and uncertainties in the current database on citrinin, the CONTAM Panel concluded that the derivation of a health based guidance value, e.g. a tolerable daily intake, was not appropriate. The Panel explored the use of a margin of exposure (MOE) approach for the risk characterisation of citrinin. However, due to the lack of data on human dietary exposure, no MOE could be calculated.

In order to give risk managers a tool to evaluate the risk of citrinin in food and feed, the Panel decided to characterise the risk of citrinin on the available data on nephrotoxicity, and determined therefore a level of no concern for nephrotoxicity. Applying a default uncertainty factor of 100 to the NOAEL of 20 µg/kg b.w. per day, accounting for inter-species variation (extrapolation from animals to humans) and for inter-individual variation (within the human population), the CONTAM Panel concluded that there would be no concern for nephrotoxicity in humans at an exposure level of 0.2 µg/kg b.w. per day. Based on the available data, a concern for genotoxicity and carcinogenicity could not be excluded at the level of no concern for nephrotoxicity.

8. Risk characterisation

8.1. Human health risk characterisation

The CONTAM Panel concluded that the available occurrence data either submitted to EFSA in response to the call for data or from the literature were not adequate to carry out a dietary exposure assessment for the general population or specific population groups. Therefore, the Panel decided to use an alternative approach and estimated the critical citrinin concentration in grains and grain-based products that would result in an exposure equal to the level of no concern for nephrotoxicity (see Section 7.6). Mean and high (95th percentile) consumption ranges of grains and grain-based products were defined through the minimum and maximum consumption across European dietary surveys (see Appendix C, Tables C1-C7). For high-consuming toddlers, other children (from 3 up to and including 9 years of age) and adults the critical citrinin concentration is in the range between 9 and 53 µg/kg grains and grain-based products and between 19 and 100 µg/kg grains and grain-based products for average consumers of these three age classes. For the other age classes (infants, adolescents, elderly and very elderly) the concentration of citrinin resulting in an exposure equal to the level of no concern for nephrotoxicity were all higher compared to those for adults (Appendix C, Table C1-C7).

In the literature, citrinin concentrations in grains and grain-based products intended for human consumption have been reported up to 420 µg/kg in grains (Table 2) and up to 42 µg/kg in grain-based products (Table 3). It should be noted that 90 % of the published occurrence data in grains intended for human consumption and 73 % of the occurrence data in grain-based products are left censored and also that targeted sampling cannot be excluded. Therefore, based on the available data, no firm conclusion can be made regarding the likelihood to exceed the level of no concern for nephrotoxicity on a daily basis over a prolonged period. In addition, there is evidence that food commodities other than grains and grain-based products can also be sources of citrinin, but the overall contribution to human exposure could not be estimated.

The CONTAM Panel noted that this risk characterisation was limited to exposure to citrinin, although combined exposures with other mycotoxins, in particular with ochratoxin A and/or aflatoxins and/or patulin in food may occur. In the absence of evidence that citrinin at low doses can exacerbate toxic effects of any other mycotoxin, the CONTAM Panel concluded that the combined effect of citrinin and ochratoxin A is at most additive.

8.2. Animal health risk characterisation

The CONTAM Panel considered *in vivo* experiments with pigs, poultry, rabbits and dogs to identify a NOAEL/LOAEL for citrinin (see Section 7.4). For pigs a NOAEL of 20 µg/kg b.w. was identified. The limited database and, in particular, the limited quality of the data available for poultry, rabbits and dogs, did not allow identification of a reliable NOAEL/LOAEL for these species. Other animals (e.g., ruminants, fish) or companion animals other than dogs could not be assessed because no data suitable for risk assessment were available.

The CONTAM Panel concluded that at present, the database describing the occurrence of citrinin in feed is too weak to carry out an exposure assessment and to assess properly the risk for farm and companion animals regarding citrinin. Therefore, the CONTAM Panel decided to base the risk characterisation of citrinin on an alternative approach and to estimate the citrinin concentration in

grains that causes an exposure equal to the NOAEL identified in Section 7.4. Using mean live weights and mean feed intake for pigs and the maximum inclusion level for grains in feed as given in Table 4 (see Section 5.2) a critical citrinin concentration in grains was calculated. For pigs (fattening pigs and sows) and piglets this critical citrinin concentration is between 640 and 1 173 µg/kg grains.

In the literature, citrinin concentrations in grains intended for animal consumption have been reported up to 998 µg/kg. It should be noted that 91 % of these published occurrence data are left censored and also that targeted sampling cannot be excluded. Therefore, it is unlikely that pigs will consume grains that exceed the critical citrinin concentration on a daily basis over a prolonged time. In addition, there is evidence that feed commodities other than grains can also be sources of citrinin, but the overall contribution to human exposure could not be estimated.

It should be noted that this risk characterisation was limited to exposure to citrinin, although combined exposures with other mycotoxins, in particular with ochratoxin A and/or aflatoxins in feed may occur. In the absence of evidence that citrinin at low doses can exacerbate toxic effects of any other mycotoxin, the CONTAM Panel concluded that the combined effect of citrinin and ochratoxin A is at most additive.

9. Uncertainty analysis

The evaluation of the inherent uncertainties in the assessment of exposure to citrinin has been performed following the guidance of the Opinion of the Scientific Committee related to Uncertainties in Dietary Exposure Assessment (EFSA, 2006). In addition, the report on 'Characterizing and Communicating Uncertainty in Exposure Assessment' has been considered (WHO/IPCS, 2008). According to the guidance provided by the EFSA opinion (2006) the following sources of uncertainties have been considered: Assessment objectives, exposure scenario, exposure model, and model input (parameters).

9.1. Assessment objectives

The objectives of the assessment were clearly specified in the terms of reference. The derivation of a health-based guidance value was not possible and the risk characterisation remained incomplete due to the lack of data on occurrence of citrinin and the limitations and uncertainties in the current toxicological database.

9.2. Exposure scenario/Exposure model

In response to EFSA's request to submit occurrence data on citrinin in food and feed, only one Member State submitted data on 30 samples. The CONTAM Panel considered that the data set reported to EFSA was not adequate for exposure assessment. Therefore, the occurrence levels of citrinin from the literature were reviewed. However, due to the limited number of studies, the lack of detailed information in publications, high percentage of left censored data and the use of different analytical methods with varying LOD/LOQ values, the CONTAM Panel concluded that the available occurrence data either from the EFSA call for data or from the literature were not adequate to carry out a dietary exposure assessment for animals as well as humans. As an alternative, the CONTAM Panel based the risk characterisation on the critical citrinin concentrations in food or feed that might lead to an exposure equal to the level of no concern for nephrotoxicity for humans or to the NOAEL derived for animals, meaning that higher citrinin concentrations on a daily basis might lead to a potential health concern. Although the occurrence data in the different food and feed groups were limited, the CONTAM Panel selected grains (intended for human and animal consumption) and grain-based products (intended for human consumption) since for this food/feed group the best available occurrence information was available and because of the relevance of this group for animal/human consumption.

Use of occurrence levels of citrinin from the literature as the only data to be used for evaluating the critical citrinin concentration is a source of considerable uncertainty.

The restriction of the current estimations to grains and grain-based products adds to the uncertainty as other food and feed commodities can contribute to citrinin exposure. The uncertainty of the sampling objectives (targeted/non-targeted sampling) of studies reported in the literature and the measurement uncertainty of analytical methods including sampling may add to the overall uncertainty of this assessment.

9.3. Uncertainties in the hazard identification and characterisation

The Panel noted that there is a large gap between the NOAEL derived from a 90-day study on rats, where all dose groups exhibited no effects on the critical endpoints of nephrotoxicity, and the citrinin concentration that cause reproducible toxic effects in experimental animals, and that data to assess adverse effects and toxicity in humans were not available.

Data on the mechanism of citrinin toxicity were limited and information on the mode of action of citrinin was exclusively available from *in vitro* studies. Genotoxicity could not be established since citrinin was not mutagenic in conventional bacterial assays but effects seen in *in vivo* studies would not exclude direct DNA reactivity of citrinin. Therefore, given the high incidence of adenomas but no evidence of carcinomas in experimental animals in a 80-week study, the CONTAM Panel concluded that there is a high uncertainty for either absence or presence of genotoxic and carcinogenic effects of citrinin in animals and/or humans.

The assessment of the available data on the interaction between citrinin and ochratoxin A or patulin did not support the existence of a synergistic interaction but the database for this conclusion is weak.

9.4. Summary of uncertainties

The CONTAM Panel evaluated the impact of uncertainties on the derived critical citrinin concentrations in food and feed. Due to the lack of exposure data, lack of sufficient toxicological dose-response information and limited information on the mode of action, the CONTAM Panel concluded that the magnitude of the overall uncertainties is large, and that the risk characterisation remained incomplete.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

General

- Citrinin is a nephrotoxic mycotoxin produced by several species of the genera *Aspergillus*, *Penicillium* and *Monascus*. Citrinin is generally formed after harvest and occurs mainly in stored grains, but also in other plant products such as beans, fruits, fruit and vegetable juices, herbs and spices, and also in spoiled dairy products. Citrinin is known to occur also in *Monascus* fermentation products, which have been used in Asia for centuries for meat preservation and food colouring.

Methods of sampling and analysis

- Instrumental techniques for citrinin analysis include fluorimetric, chromatographic and immunochemical techniques. To date, high performance liquid chromatography with fluorescence detection (HPLC-FLD) is the method of choice for routine citrinin analysis, but rigorous sample clean-up methods are needed to obtain methods with low limits of detection (LODs). Up to now, LODs in the range of 0.1 to 10 µg/kg are reported for HPLC-FLD.

- One of the major challenges in citrinin analysis relates to its instability in various organic solvents and at higher temperatures.

- So far, none of the applied analytical methods have been validated by inter-laboratory studies. In addition, no certified reference materials or proficiency tests are available for the determination of citrinin in food or feed.

Occurrence and effect of processing

- Following a call from the European Food Safety Authority (EFSA) for data, analytical results from only 30 samples were submitted by one Member State, covering the period from 2006 to 2008.

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

  - The number of investigations dealing with occurrence of citrinin in food and feed commodities is small as compared to studies concerning other mycotoxins.

  - The reported citrinin concentrations varied widely in grains both when intended for food (up to 420 µg/kg) and for feed (up to 998 µg/kg) consumption.

  - In food other than grains, the reported citrinin concentrations varied up to 42 µg/kg in grain-based products, up to 355 µg/kg in herbs and up to 0.2 µg/L in fruit and vegetable juices. No quantitative data were identified for fruits.

  - The reported citrinin concentrations in feed other than grains also varied widely (up to > 405 µg/kg).

  - A high proportion (up to > 90 %) of left censored data (below LOD or limit of quantification (LOQ)) were observed. In the case of positive results the impact of targeted versus non-targeted sampling could not be assessed.

  - In both food and feed, co-occurrence of citrinin with other mycotoxins is observed, especially with ochratoxin A in grains and grain-based products and with patulin in fruits and fruit and vegetable juices.

- Citrinin is heat sensitive and decomposes during heat treatment to form other complex compounds, such as citrinin H_1 and citrinin H_2, respectively with higher and weaker cytotoxicity than the original citrinin.

Human exposure

- The available occurrence data either submitted to EFSA in response to the call for data or from the literature were not adequate to carry out human dietary exposure assessments for the general population or specific population groups.

Animal exposure

- The available occurrence data either submitted to EFSA in response to the call for data or from the literature were not adequate to carry out animal dietary exposure assessments.

Hazard identification and characterisation

Toxicokinetics

- Specific toxicokinetic studies with oral administration are not available for citrinin.

- Experimental data indicate that citrinin residues may occur in edible tissues and eggs following oral exposure of animals with highly contaminated feed materials.

Toxicity

- Acute oral lethal doses (LD_{50}) in mice and rabbits are of the order of 100 mg/kg body weight (b.w.).

- One subchronic dietary study (90 days) in rats showed no adverse effects at a citrinin dose of 20 µg/kg b.w. per day in feed, the highest dose tested in this study.

- The kidney was identified as the principal target organ for citrinin. This was confirmed also in a long-term study (80 weeks) with citrinin at 1 000 mg/kg in the diet of rats, showing progressive histopathological changes and high incidences of adenomas in the kidneys.

- Studies on immunotoxicity of citrinin do not allow a conclusive evaluation.

- *In vitro* and *in vivo* studies provided clear evidence for reproductive toxicity and, teratogenic and embryotoxic effects of citrinin.

    - The doses tested in the *in vivo* experiments exerted clear signs of maternal toxicity, including nephrotoxicity, indicating that the teratogenic effects might be secondary to maternal toxicity.

    - One study in male mice showed adverse effects on male reproductive organs and sperm quality when citrinin was given intraperitoneally.

- Citrinin is not mutagenic in conventional bacterial assays with or without metabolic activation, but induces micronuclei, aneuploidy, and chromosomal aberrations *in vitro* in mammalian cells. *In vivo* it induced chromosome abnormalities and hypodiploidy in the bone marrow of mice.

- As no life-time exposure studies are available, no conclusion can be drawn regarding the potential carcinogenicity of citrinin.

- From the data available on mammalian cells in culture, the CONTAM Panel concluded that citrinin toxicity is exerted via multiple pathways such as DNA and RNA synthesis inhibition, inhibition of microtubule assembly and of tubulin polymerization, alteration of mitochondrial functionality with consequent increase in reactive oxygen species production, inactivation of the shock protein 90 (HSP90) multichaperone complex and activation of the signal transduction pathway and the caspase-cascade system that results in apoptotic cell death.

- The available evidence indicates that citrinin at low doses does not exacerbate the toxic effects of other mycotoxins. The CONTAM Panel concluded that the combined effect of citrinin and ochratoxin A is at most additive.

Adverse effects in livestock and pet animals

- Only few studies on adverse effects of citrinin in pigs, rabbits, poultry and dogs could be identified.

- No effect was reported in a study with pigs given 20 µg citrinin/kg b.w. per day. The CONTAM Panel considered this intake value as a no-observed-adverse-effect level (NOAEL), which is consistent with the results from a subchronic study in rodents.

- In poultry, the reported effects varied widely in type and severity of effects depending on species, age of the birds and the design of the studies. As further details were missing, these results were not suitable for risk characterisation and no NOAEL/lowest-observed-adverse-effect level (LOAEL) for poultry could be identified.

- In rabbits, moderate health effects were reported when citrinin was administered via the feed (15 mg/kg feed) for 60 days. However, no intake value could be calculated and no NOAEL/LOAEL for rabbits could be identified.

- The available data for dogs could not be used for risk assessment due to co-exposure with ochratoxin A. No other data for companion animals could be identified.

- Experimental data regarding systemic effects in ruminants are not available. It is assumed that citrinin is highly degraded and metabolised through the microbial activity in the forestomachs of ruminants. However, an impairment of the rumen flora due to the antibacterial effect of citrinin cannot be excluded.

Level of no concern for nephrotoxicity

- Based on a subchronic study (90 days) in rats, the CONTAM Panel identified a NOAEL of 20 µg/kg b.w. per day for nephrotoxicity. Since no higher doses were used in this study, it does not provide information on adverse effect levels.

- The Panel concluded that due to the magnitude of the uncertainties, the derivation of a health-based guidance value for citrinin was not appropriate.

- For compounds that may be genotoxic and carcinogenic, EFSA recommends the use of a margin of exposure (MOE) approach for risk characterisation. However, due to the lack of data on human dietary exposure, no MOE could be calculated.

- The Panel decided to characterise the risk of citrinin on the available data on nephrotoxicity and determined a level of no concern for nephrotoxicity. Applying an uncertainty factor of 100 to the NOAEL of 20 µg/kg b.w. per day results in a level of no concern for nephrotoxicity in humans of 0.2 µg/kg b.w. per day. Based on the available data, a concern for genotoxicity and carcinogenicity could not be excluded at the level of no concern for nephrotoxicity.

Human risk characterisation

- In the absence of adequate exposure data, characterisation of the risk of citrinin as a food contaminant was based on the estimate of the critical citrinin concentrations in grains and grain-based products that would result in an exposure equal to the level of no concern for nephrotoxicity using human consumption data.

- There is evidence that food commodities other than grains and grain-based products can also be sources of citrinin, but the overall contribution to human exposure could not be estimated.

- For high consuming toddlers, other children (from 3 up to and including 9 years of age) and adults the critical citrinin concentration ranges between 9 and 53 µg/kg grains and grain-based products and between 19 and 100 µg/kg grains and grain-based products for average consumers of these age classes.

- Based on the available data no firm conclusion can be made regarding the likelihood of exceeding the level of no concern for nephrotoxicity on a daily basis over a prolonged period.

Animal risk characterisation

- In the absence of an exposure assessment, the risk characterisation remained incomplete and was based on the estimate of a critical citrinin concentration in grains that would result in exposure equal to the identified NOAEL.

- There is evidence that feed commodities other than grains can also be sources of citrinin, but the overall contribution to animal exposure cannot be estimated.

- The critical citrinin concentration resulting in an exposure equal to the NOAEL ranges between 640 and 1 173 µg/kg grains for pigs. It is unlikely that pigs will consume grains that exceed the critical citrinin concentration on a daily basis over a prolonged time.

RECOMMENDATIONS

- There is a need for more data regarding the occurrence of citrinin in food and feed in Europe.

- There is a need for certified reference materials and defined performance criteria for the analysis of citrinin in food and feed.

- There is a need for well-designed toxicological studies in laboratory animal species to further explore the toxicological potential of citrinin and to characterize the dose-response relationships.

- There is a need for more data on farm animal toxicity and the carry over of citrinin from the feed to animal products intended for human consumption.

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APPENDICES

A. CITRININ PRODUCING FUNGI

Recently, citrinin biosynthesis in fungi has been discussed only for several species in the genera *Aspergillus* and *Penicillium*. However, isolates of *Monascus* spp. are now also recognised as producers of citrinin (Blanc et al., 1995a,b). Table A1 gives an overview of the current identity of fungi that are apparently able to produce citrinin. Due to considerable revisions in taxonomy, particularly within the genus *Penicillium* (Samson and Frisvad, 2004), and difficulties in correct species assignation to isolates within that genus, this identity has changed during the years.

Besides citrinin, some of the fungi listed in Table A1 are also able to produce other mycotoxins, namely ochratoxin A and patulin

Table A1: Current identity of fungi with confirmed ability to produce citrinin (based on Balajee et al., 2009; Samson and Frisvad, 2004; Frisvad, personal communication; Udagawa and Baba, 1998).

Aspergillus alabamensis sp.nov.
Aspergillus carneus (Tiegh.)Blochwicz
Aspergillus niveus Blochwicz
Monascus aurantiacus Zhong Q. Li
Monascus floridanus P.F. Cannon & E.L. Barnard
Monascus lunisporas sp.nov.
Monascus pallens P.F. Cannon, Abdullah & B.A. Abbas
Monascus pilosus K. Sato ex D. Hawkesw. & Pitt
Monascus purpureus Went
Monascus ruber Tiegh.
Monascus sanguineus P.F. Cannon, Abdullah & B.A. Abbas
Penicillium chrzaszczii K.M. Zalessky
Penicillium citrinum Thom
Penicillium decaturense S.W. Peterson, E.M. Bayer & Wicklow
Penicillium expansum Link
Penicillium gorlenkoanum Baghd.
Penicillium hetheringtonii Houbraken, Frisvad & Samson
Penicillium manginii Duche & R.Heim
Penicillium miczynskii K.M. Zalessky
Penicillium odoratum M. Chr.& Backus
Penicillium radicicola Overy & Frisvad
Penicillium verrucosum Dierckx
Penicillium westlingii K.M. Zalessky

Citrinin has also been reported as a product of the fungi *Pythium ultimum* (Endo and Kuroda, 1976) and *Clavariopsis aquatica* (Broadbent, 1966) but neither has since been confirmed. Similarly, an old report of a plant source, the Australian legume *Crotolaria crispata* (Ewart, 1933) needs verification or recognition that a fungal endophyte could be the primary source. A recent report of ochratoxinogenic Penicillia as endophytes in coffee plants (Vega et al., 2006) might offer analogy here.

B. OVERVIEW OF PREVIOUSLY REPORTED LITERATURE DATA ON OCCURRENCE OF CITRININ

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

Commodity	Country of origin	Year [a]	n	LOD (µg/kg)	LOQ (µg/kg)	Analytical method	% LC	Descriptive statistics (µg/kg)[g]				Reported levels (µg/kg)	Co-occurrence with OTA, AF and PAT[e]	Reference
								Min	P50	Max	Mean			
Europe														
Wheat (feed)	Russia	2003-2006	243	10	n.r.	ELISA	96	50	n.r.	144	n.r.	n.r.	n.r.	Kononenko and Burkin, 2008
Barley (feed)	Russia	2003-2006	138	10	n.r.	ELISA	95	63	n.r.	998	n.r.	n.r.	n.r.	Kononenko and Burkin, 2008
Maize (feed)	Russia	2003-2006	157	10	n.r.	ELISA	98	218	830	953	n.r.	218; 830; 953	n.r.	Kononenko and Burkin, 2008
Wheat (food)	Bulgaria	1998	37	5	n.r.	ELISA	92	20	83	420	n.r.	20; 83; 420[c]	CIT + OTA: 2 (5 %)	Vrabcheva et al., 2000
Maize (food)	Bulgaria	1998	23	5	n.r.	ELISA	100	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	Vrabcheva et al., 2000
Barley (feed)	Bulgaria	1998	6	5	n.r.	ELISA	100	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	Vrabcheva et al., 2000
Oats(feed)	Bulgaria	1998	9	5	n.r.	ELISA	100	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	Vrabcheva et al., 2000
Wheat (feed)	Romania	1997	25	200	n.r.	ELISA	100	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	Curtui et al., 1998
Maize (feed)	Romania	1997	30	200	n.r.	ELISA	97	n.a.	n.a.	n.a.	n.a.	580	n.r.	Curtui et al., 1998
Durum wheat	Switzerland	n.r.	4	0.1	n.r.	HPLC	50	0.3	/	0.7	n.r.	0.3; 0.7	n.r.	Dick et al., 1988
Cereals (wheat, barley and oats) for feed use[b]	UK	n.r.	46	n.r.	n.r.	TLC and HPLC	43	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	Scudamore and Hetmanski, 1995
Barley (feed ingredient)	UK	1992	45	1	n.r.	HPLC	84	n.r.	n.r.	8	n.r.	n.r.	OTA+CIT: 3 (7 %) OTA+ CIT: 1 (2 %)	Scudamore et al., 1997
Wheat (feed ingredient)	UK	1992	50	1	n.r.	HPLC	70	n.r.	n.r.	10	n.r.	n.r.	OTA+CIT: 5 (10 %)	Scudamore et al., 1997
Maize	Bulgaria	1987	44	15-20	n.r.	TLC	70	100	n.r.	1 500	n.r.	n.r.	n.r.	Petkova-Bocharova et al., 1991
Maize	Bulgaria	1988	68	15-20	n.r.	TLC	78	50	n.r.	1 000	n.r.	n.r.	n.r.	Petkova-Bocharova et al., 1991
Maize	Bulgaria	1989	55	15-20	n.r.	TLC	69	50	n.r.	1 100	n.r.	n.r.	n.r.	Petkova-Bocharova et al., 1991
Maize	Bulgaria	1990	65	15-20	n.r.	TLC	78	50	n.r.	1 000	n.r.	n.r.	n.r.	Petkova-Bocharova et al., 1991
Wheat (food)	Czeck Republic	2005	1	0.5	1.5	HPLC	0	n.a.	n.a.	n.a.	n.a.	< LOQ	CIT + OTA: 1 (100 %)	Polisenska et al., 2010

Table B1: Continued.

Commodity	Country of origin	Year [(a)]	n	LOD (µg/kg)	LOQ (µg/kg)	Analytical method	% LC	Descriptive statistics (µg/kg)[(g)]				Reported levels (µg/kg)	Co-occurrence with OTA, AF and PAT[(e)]	Reference
								Min	P50	Max	Mean			
Wheat (food)	Czeck Republic	2006	9	0.5	1.5	HPLC	100	n.a.	n.a.	n.a.	n.a.	n.a.	CIT + OTA: 0 OTA: 1 (11 %)	Polisenska et al., 2010
Wheat (food)	Czeck Republic	2007	1	0.5	1.5	HPLC	100	n.a.	n.a.	n.a.	n.a.	n.a.	CIT + OTA: 0 OTA:1 (100 %)	Polisenska et al., 2010
Wheat (feed)	Czeck Republic	2006	1	0.5	1.5	HPLC	100	n.a.	n.a.	n.a.	n.a.	n.a.	CIT + OTA: 0 OTA:0	Polisenska et al., 2010
Wheat (feed)	Czeck Republic	2007	10	0.5	1.5	HPLC	100	n.a.	n.a.	n.a.	n.a.	n.a.	CIT + OTA: 0 OTA: 2 (20 %)	Polisenska et al., 2010
Barley (food)	Czeck Republic	2007	3	0.5	1.5	HPLC	33[(h)]	1.82	n.a.	93.64[(f)]	n.r.	1.82; 93.64[(d)]	CIT + OTA: 2 (67 %) OTA: 0	Polisenska et al., 2010
Barley (food)	Czeck Republic	2008	1	0.5	1.5	HPLC	100[(i)]	n.a.	n.a.	n.a.	n.a.	n.a.	CIT + OTA: 1 (100 %) OTA: 0	Polisenska et al., 2010
Barley (feed)	Czeck Republic	2007	6	0.5	1.5	HPLC	66[(i)]	5.25	n.a.	13.17	n.r.	5.25; 13.17[(d)]	CIT + OTA: 3 (50 %) OTA: 1 (17 %)	Polisenska et al., 2010
North America														
Cereals (wheat, oats, barley, rye)	Canada	1968	29	n.r.	n.r.	TLC	55	70	n.r.	80 000	n.r.	70; 100; 280; 960; 1 000; 1 700; 2 000; 2 100; 2 400; 6 700; 10 000; 60 000; 80 000	CIT + OTA: 12 (57 %) OTA: 3 (14 %)	Scott et al., 1972
Asia														
Cereals (rice, barley, maize, buckwheat) (food use)	Japan	1980	27	n.r.	n.r.	TLC	100	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	Nishijima, 1984
Maize	India	1994-1997	197	n.r.	n.r.	TLC	99.5	n.a.	n.a.	n.a.	n.a.	12	n.r.	Janardhana et al., 1999
Wheat (food)	Japan	n.r.	12	n.r.	0.1	LC-MS/MS	92	n.a.	n.a.	n.a.	n.a.	0.19	OTA+CIT+DON: 1 (8 %)	Tabata et al., 2008
Barley (food)	Japan	n.r.	3	n.r.	0.1	LC-MS/MS	100	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	Tabata et al., 2008
Oat (food)	Japan	n.r.	3	n.r.	0.1	LC-MS/MS	100	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	Tabata et al., 2008
Rye (food)	Japan	n.r.	1	n.r.	0.1	LC-MS/MS	100	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	Tabata et al., 2008
Coix seed (food)	Japan	n.r.	2	n.r.	0.1	LC-MS/MS	100	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	Tabata et al., 2008
Rice (food)	Japan	n.r.	5	n.r.	0.1	LC-MS/MS	100	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	Tabata et al., 2008
Buckwheat (food)	Japan	n.r.	2	n.r.	0.1	LC-MS/MS	0	0.55	n.a.	0.62	n.r.	0.55; 0.62	OTA+CIT: 2 (100 %)	Tabata et al., 2008

Table B1: Continued.

Commodity	Country of origin	Year [(a)]	n	LOD (µg/kg)	LOQ (µg/kg)	Analytical method	% LC	Descriptive statistics (µg/kg)[(g)]				Reported levels (µg/kg)	Co-occurrence with OTA, AF and PAT[(e)]	Reference
								Min	P50	Max	Mean			
Maize (food)	Japan	n.r.	3	n.r.	0.1	LC-MS/MS	100	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	Tabata et al., 2008
Maize (food)	India	1981	30	n.r.	n.r.	TLC	100	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	Reddy et al., 1983
Sorghum (food)	India	1981	20	n.r.	n.r.	TLC	100	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	Reddy et al., 1983
Ragi (food)	India	1981	37	n.r.	n.r.	TLC	100	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	Reddy et al., 1983
Broken rice (food)	India	1981	32	n.r.	n.r.	TLC	100	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	Reddy et al., 1983
Parboiled rice (food)	India	1981	18	n.r.	n.r.	TLC	67	12	n.r.	55	n.r.	n.r.	No co-occurrence with AFB1, AFB2, OTA	Reddy et al., 1983
Rice	Vietnam	n.r.	100	0.11	0.35	HPLC	87	n.r.	n.r.	0.42	0.38	n.r.	Co-occurrence with AFB1 and OTA	Nguyen et al., 2007
Africa														
White maize	Egypt	1991-1993	27	n.r.	n.r.	n.r.	100	/	/	/	/	/	n.r.	El-Sayed, 1996
Yellow maize	Egypt	1991-1993	36	n.r.	n.r.	n.r.	92	71.2	n.r.	211.3	62.9	n.r.	n.r.	El-Sayed, 1996
Wheat	Egypt	1991-1993	26	n.r.	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	n.a.	n.r.	El-Sayed, 1996
Barley	Egypt	1991-1993	27	n.r.	n.r.	n.r.	44	53.2	n.r.	100.0	64.4.	n.r.	n.r.	El-Sayed, 1996
Rice	Egypt	1991-1993	33	n.r.	n.r.	n.r.	61	6.41	n.r.	27.9	13.8	n.r.	n.r.	El-Sayed, 1996
Cereals (sorghum, sorghum malt, maize grits, spent) (used for brewing beer)	South Africa	n.r.	30	n.r.	n.r.	TLC	100	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	Odhav and Naiker, 2002
Maize (white and yellow)	Egypt	n.r.	20	n.r.	n.r.	TLC and HPLC	95	n.a.	n.a.	n.a.	n.a.	300	n.r.	Aziz et al., 2006
Soybean	Egypt	n.r.	10	n.r.	n.r.	TLC and HPLC	80	130	/	270	n.r.	130; 270	n.r.	Aziz et al., 2006
Wheat	Egypt	n.r.	10	n.r.	n.r.	TLC and HPLC	90	n.a.	n.a.	n.a.	n.a.	112	n.r.	Aziz et al., 2006

Table B1: Continued.

Commodity	Country of origin	Year [(a)]	n	LOD (µg/kg)	LOQ (µg/kg)	Analytical method	% LC	Descriptive statistics (µg/kg)[(g)]				Reported levels (µg/kg)	Co-occurrence with OTA, AF and PAT[(e)]	Reference
								Min	P50	Max	Mean			
Barley	Egypt	n.r.	10	n.r.	n.r.	TLC and HPLC	90	n.a.	n.a.	n.a.	n.a.	100	n.r.	Aziz et al., 2006
Rice (shelled and unshelled)	Egypt	n.r.	20	n.r.	n.r.	TLC and HPLC	100	n.a.	n.a.	n.a.	n.a.	n.a.	n.r.	Aziz et al., 2006
Paddy rice	Egypt	2002	30	n.r.	n.r.	fluorometer	67	2.74	12.78	28.54	n.r.	2.74; 4.46; 5.48; 5.82; 12.7; 12.86; 16.26; 19.74; 21.36; 28.54	n.r.	Abd-Allah and Ezzat, 2005

n.a.: not applicable; n.r.: not reported; LC: left censored data (values below the LOD or LOQ); LOD: Limit of detection; LOQ: Limit of quantification; Min: minimum; Max: maximum; P50: 50th percentile; UK: United Kingdom; ELISA: Enzyme linked immunosorbent assay; HPLC: High-performance liquid chromatography; TLC: Thin-layer chromatography; CIT: Citrinin; OTA: ochratoxin A, AFB1: Aflatoxin B_1; AFB2: Aflatoxin B_2.
(a): Year of sample collection.
(b): considered to be representative of poorly stored cereals. Several samples were obtained from feed-related incidents.
(c): reported values are not corrected for recovery.
(d): reported values are corrected for recovery.
(e): number of samples (% of samples) in which the mentioned mycotoxins occurred together.
(f): sample was offered to a malt house but not accepted due to a higher content of admixtures and impurities and a mouldy smell.
(g): descriptive statistics of numerical data (above LOD or LOQ) as reported by the authors.
(h): 2 samples above LOQ and 1 sample above LOD but below LOQ.
(i): 1 sample above LOD but below LOQ.
(j): 2 samples above LOQ and 1 sample above LOD but below LOQ.

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

Commodity	Origin	Year [a]	n	LOD (µg/kg)	LOQ (µg/kg)	Analytical method	% LC	Descriptive statistics (µg/kg) [e]				Reported levels (µg/kg)	Co-occurrence with OTA, AF, PAT[c]	Reference
								Min	P50	Max	Mean			
Grain-based products														
Europe														
Flour	Switzerland	n.r.	21	0.1	n.r.	HPLC	48	0.2	0.6	1.0	n.r.	0.2 (2x); 0.3; 0.5; 0.6 (3x); 0.7 (3x); 1.0	n.r.	Dick et al., 1988
Pasta	Switzerland	n.r.	2	0.1	n.r.	HPLC	50	n.a.	n.a.	n.a.	n.a.	0.5	n.r.	Dick et al., 1988
Wheat bran	Switzerland	n.r.	5	0.1	n.r.	HPLC	100	n.a.	n.a.	n.a.	n.a.	n.a.	n.r.	Dick et al., 1988
Breakfast cereals	France	n.r.	45	0.5	1.5	HPLC	82	<1.5	/	42	n.r.	<LOQ (2x); 5; 7; 12 (2x); 19; 42	OTA+CIT: 8 (18 %)	Molinié et al., 2005
Mouldy bread	England	n.r.	50	n.r.	n.r.	TLC	100	n.a.	n.a.	n.a.	n.a.	n.a.	n.r.	Osborne, 1980
Mouldy flour	England	n.r.	7	n.r.	n.r.	TLC	86	n.a.	n.a.	n.a.	n.a.	traces	n.r.	Osborne, 1980
Asia														
Flour (wheat, rice, buckwheat, maize, rye)	Japan	1980	31	n.r.	n.r.	TLC	94	27	/	73	n.r.	27; 73	AFB1+AFB2+CIT: 2 (6 %)	Nishijima, 1984
Maize soup	Japan	1980	3	n.r.	n.r.	TLC	100	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	Nishijima, 1984
Maize starch	Japan	1980	1	n.r.	n.r.	TLC	100	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	Nishijima, 1984
Maize flakes	Japan	1980	2	n.r.	n.r.	TLC	100	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	Nishijima, 1984
Africa														
Wheat flour	Egypt	1991-1993	31	n.r.	n.r.	TLC	100	n.a.	n.a.	n.a.	n.a.	n.a.	n.r.	El-Sayed, 1996
Fermented maize dough	Ghana	n.r.	20	0.1	n.r.	HPLC	0	0.7	n.r.	585	99	n.r.	Co-occurrence of CIT with AF and OTA	Kpodo et al., 1996
Beans and peas														
Europe														
Beans	Bulgaria	1987	44	15-20	n.r.	TLC	77	50	n.r.	1 000	n.r.	n.r.	n.r.	Petkova-Bocharova et al., 1991
Beans	Bulgaria	1988	68	15-20	n.r.	TLC	78	20	n.r.	380	n.r.	n.r.	n.r.	Petkova-Bocharova et al., 1991
Beans	Bulgaria	1989	55	15-20	n.r.	TLC	73	50	n.r.	800	n.r.	n.r.	n.r.	Petkova-Bocharova et al., 1991
Beans	Bulgaria	1990	65	15-20	n.r.	TLC	80	20	n.r.	800	n.r.	n.r.	n.r.	Petkova-Bocharova et al., 1991
Asia														
Beans	Japan	1980	4	n.r.	n.r.	TLC	100	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	Nishijima, 1984

Table B2: Continued.

Commodity	Origin	Year [a]	n	LOD (µg/kg)	LOQ (µg/kg)	Analytical method	% LC	Descriptive statistics (µg/kg) [e]				Reported levels (µg/kg)	Co-occurrence with OTA, AF, PAT[c]	Reference
								Min	P50	Max	Mean			
Africa														
Beans	Egypt	1991-1993	42	n.r.	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	n.a.	n.r.	El-Sayed, 1996
Peas	Egypt	1991-1993	32	n.r.	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	n.a.	n.r.	El-Sayed, 1996
Kidney beans	Egypt	n.r.	10	n.r.	n.r.	TLC and HPLC	90	n.a.	n.a.	n.a.	n.a.	370	n.r.	Aziz et al., 2006
Fruits, fruit juices and vegetable juices														
Europe														
Apples (some presenting visible fungal infection)	Croatia	n.r.	100	n.r.	n.r.	TLC	81	50	n.r.	240	n.r.	n.r.	n.r.	Pepeljnjak et al., 2002
Apples with rotten spots	Portugal	n.r.	351	15-20	n.r.	TLC	76	n.r.	n.r.	n.r.	n.r.	n.r.	CIT+PAT: 69 (20 %) PAT: 241 (69 %) CIT: 14 (4 %)	Martins et al., 2002
Naturally infected fruits, juices and pulps	Denmark	n.r.	6	n.r.	n.r.	HPLC	100	n.a.	n.a.	n.a.	n.a.	n.a.	PAT: 2 (33 %)	Andersen et al., 2004
Apple juices	Germany	n.r.	35	0.08[d]	n.r.	ELISA	97	n.a.	n.a.	n.a.	n.a.	0.13[d]	n.r.	Dietrich et al., 1999b
Tomato juices	Germany	n.r.	11	0.08[d]	n.r.	ELISA	91	n.a.	n.a.	n.a.	n.a.	0.12 [d]	n.r.	Dietrich et al., 1999b
Cherry juices	Germany	n.r.	5	0.08[d]	n.r.	ELISA	80	n.a.	n.a.	n.a.	n.a.	0.10 [d]	n.r.	Dietrich et al., 1999b
Black currant juices	Germany	n.r.	2	0.08[d]	n.r.	ELISA	50	n.a.	n.a.	n.a.	n.a.	0.20 [d]	n.r.	Dietrich et al., 1999b
Grape juices	Germany	n.r.	2	0.08[d]	n.r.	ELISA	100	n.a.	n.a.	n.a.	n.a.	n.a.	n.r.	Dietrich et al., 1999b
Apples (naturally rotted)	Portugal	n.r.	30	30-40	n.r.	TLC	73	300	n.r.	3 000	n.r.	n.r.	5 samples positive for CIT are also positive for AFB1	Gimeno and Martins, 1983
North America														
Apples (naturally rotten)	Canada	n.r.	61	n.r.	n.r.	TLC	97	n.r.	n.r.	n.r.	n.r.	traces	CIT+PAT: 2 (3 %)	Harwig et al., 1973

Table B2: Continued.

Commodity	Origin	Year [a]	n	LOD (µg/kg)	LOQ (µg/kg)	Analytical method	% LC	Descriptive statistics (µg/kg) [e]				Reported levels (µg/kg)	Co-occurrence with OTA, AF, PAT[c]	Reference
								Min	P50	Max	Mean			
Africa														
Strawberry[b]	Egypt	n.r.	10	40-100	n.r.	TLC	100	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	Azziz and Moussa, 2002
Apricot[b]	Egypt	n.r.	10	40-100	n.r.	TLC	100	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	Azziz and Moussa, 2002
Plum[b]	Egypt	n.r.	10	40-100	n.r.	TLC	100	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	Azziz and Moussa, 2002
Peach[b]	Egypt	n.r.	10	40-100	n.r.	TLC	100	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	Azziz and Moussa, 2002
Grape[b]	Egypt	n.r.	10	40-100	n.r.	TLC	80	n.a.	n.a.	n.a.	n.a.	70 (2x)	n.r.	Azziz and Moussa, 2002
Date[b]	Egypt	n.r.	10	40-100	n.r.	TLC	100	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	Azziz and Moussa, 2002
Fig[b]	Egypt	n.r.	10	40-100	n.r.	TLC	90	n.a.	n.a.	n.a.	n.a.	60	n.r.	Azziz and Moussa, 2002
Apple	Egypt	n.r.	10	40-100	n.r.	TLC	100	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	Azziz and Moussa, 2002
Pear[b]	Egypt	n.r.	10	40-100	n.r.	TLC	90	n.a.	n.a.	n.a.	n.a.	50	n.r.	Azziz and Moussa, 2002
Mulberry[b]	Egypt	n.r.	10	40-100	n.r.	TLC	100	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	Azziz and Moussa, 2002
Other products														
Europe														
Medicinal and aromatic herbs	Spain	n.r.	84	16.5	n.r.	ELISA	39	n.r.	n.r.	355	n.r.	n.r.	CIT co-occurred with OTA, AFs	Santos et al., 2009
Nuts and sunflower seeds	Spain	1986-1987	168	n.r.	n.r.	TLC	100	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	Jiménez et al., 1991
Mould spoiled cheeses	UK	1980	25	n.r.	n.r.	TLC and HPLC	92	n.r.	n.r.	50	n.r.	n.r.	n.r.	Jarvis, 1983
Mould spoiled cheeses	UK	1981-1982	19	n.r.	n.r.	TLC and HPLC	21	n.r.	n.r.	50	n.r.	n.r.	n.r.	Jarvis, 1983
Cheeses	Germany	n.r.	49	20	n.r.	TLC and HPLC	100	/	/	/	/	/	OTA: 0	Nowotny et al., 1983
Beers	Italy	n.r.	24	1[d]	n.r.	TLC	100	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	Cerutti et al., 1987
Asia														
Seeds in medicinal plants	India	n.r.	60	n.r.	n.r.	TLC	82	10	n.r.	760	n.r.	n.r.	n.r.	Roy and Kumari, 1991
Almond	Saudi-Arabia	n.r.	5	n.r.	n.r.	TLC	100	n.a.	n.a.	n.a.	n.a.	n.a.	n.r.	Abdel-Gawad and Zohri, 1993

Table B2: Continued.

Commodity	Origin	Year [(a)]	n	LOD (µg/kg)	LOQ (µg/kg)	Analytical method	% LC	Descriptive statistics (µg/kg) [(e)]				Reported levels (µg/kg)	Co-occurrence with OTA, AF, PAT[(c)]	Reference
								Min	P50	Max	Mean			
Cashew nut	Saudi-Arabia	n.r.	5	n.r.	n.r.	TLC	100	n.a.	n.a.	n.a.	n.a.	n.a.	n.r.	Abdel-Gawad and Zohri, 1993
Chestnut	Saudi-Arabia	n.r.	5	n.r.	n.r.	TLC	100	n.a.	n.a.	n.a.	n.a.	n.a.	n.r.	Abdel-Gawad and Zohri, 1993
Hazelnut	Saudi-Arabia	n.r.	5	n.r.	n.r.	TLC	100	n.a.	n.a.	n.a.	n.a.	n.a.	n.r.	Abdel-Gawad and Zohri, 1993
Pistachio nut	Saudi-Arabia	n.r.	5	n.r.	n.r.	TLC	100	n.a.	n.a.	n.a.	n.a.	n.a.	n.r.	Abdel-Gawad and Zohri, 1993
Walnut	Saudi-Arabia	n.r.	5	n.r.	n.r.	TLC	100	n.a.	n.a.	n.a.	n.a.	n.a.	n.r.	Abdel-Gawad and Zohri, 1993
Turmeric	India	1984-1986	9	n.r.	n.r.	TLC	78	n.a.	n.a.	n.a.	n.a.	48; 52	n.a.	Saxena and Mehrotra, 1989
Coriander	India	1984-1986	9	n.r.	n.r.	TLC	89	n.a.	n.a.	n.a.	n.a.	34	n.a.	Saxena and Mehrotra, 1989
Fennel	India	1984-1986	9	n.r.	n.r.	TLC	79	n.a.	n.a.	n.a.	n.a.	28; 59	n.a.	Saxena and Mehrotra, 1989
Cinnamon	India	1984-1986	6	n.r.	n.r.	TLC	100	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	Saxena and Mehrotra, 1989
Black pepper	India	1984-1986	8	n.r.	n.r.	TLC	88	n.a.	n.a.	n.a.	n.a.	50	n.a.	Saxena and Mehrotra, 1989
Cardamom	India	1984-1986	6	n.r.	n.r.	TLC	83	n.a.	n.a.	n.a.	n.a.	25	n.a.	Saxena and Mehrotra, 1989
Greater cardamom	India	1984-1986	6	n.r.	n.r.	TLC	100	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	Saxena and Mehrotra, 1989
Indian cassia	India	1984-1986	6	n.r.	n.r.	TLC	100	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	Saxena and Mehrotra, 1989
Ammi	India	1984-1986	7	n.r.	n.r.	TLC	100	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	Saxena and Mehrotra, 1989
Cumin	India	1984-1986	8	n.r.	n.r.	TLC	100	n.a.	n.a.	n.a.	n.a.	22	n.a.	Saxena and Mehrotra, 1989
Chili	India	1984-1986	9	n.r.	n.r.	TLC	100	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	Saxena and Mehrotra, 1989
Yellow mustard	India	1984-1986	6	n.r.	n.r.	TLC	100	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	Saxena and Mehrotra, 1989

Table B2: Continued.

Commodity	Origin	Year [(a)]	n	LOD (µg/kg)	LOQ (µg/kg)	Analytical method	% LC	Descriptive statistics (µg/kg) [(e)]				Reported levels (µg/kg)	Co-occurrence with OTA, AF, PAT[(c)]	Reference
								Min	P50	Max	Mean			
Indian mustard	India	1984-1986	7	n.r.	n.r.	TLC	100	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	Saxena and Mehrotra, 1989
Garlic	India	1984-1986	6	n.r.	n.r.	TLC	100	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	Saxena and Mehrotra, 1989
Clove	India	1984-1986	6	n.r.	n.r.	TLC	100	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	Saxena and Mehrotra, 1989
Coconut products	India	1982-1983	384	n.r.	n.r.	TLC	99	10	n.r.	60	n.r.	10; 20; 30; 60	OTA+CIT: 1 (0.26 %) OTA+AFB1: 1 (0.26 %)	Kumari and Nusrath, 1987
Black olives	Turkey	2000-2001	69	n.r	n.r.	TLC	23	n.r.	n.r.	350	n.r.	n.r.	n.r.	Heperkan et al., 2006
Africa														
Peanut	Egypt	n.r.	10	n.r.	n.r.	TLC and HPLC	100	n.a.	n.a.	n.a.	n.a.	n.a.	n.r.	Aziz et al., 2006
Hazelnut	Egypt	n.r.	20	n.r.	n.r.	TLC	100	n.a.	n.a.	n.a.	n.a.	n.a.	n.r.	Abdel-Hafez and Saber, 1993
Walnut	Egypt	n.r.	20	n.r.	n.r.	TLC	100	n.a.	n.a.	n.a.	n.a.	n.a.	n.r.	Abdel-Hafez and Saber, 1993
Spices	Egypt	n.r.	120	n.r.	n.r.	TLC	98	8	n.a.	12	n.r.	8; 12	n.r.	El-Kady et al., 1995
Commercial beers	South Africa	n.r.	6	n.r.	n.r.	TLC	100	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	Odhav and Naiker, 2002
Home-brewed beers	South Africa	n.r.	29	n.r.	n.r.	TLC	100	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	Odhav and Naiker, 2002
Black olives	Morocco	n.r.	10	0.2	0.5	HPLC	20	0.2	n.r.	0.5	n.r.	0.2 (3x); < 0.5 (2x); 0.5 (3x)	OTA+AF+CIT: 4 (40 %) OTA+CIT: 4 (40 %)	El Adlouni et al., 2006
Coffee beans	Egypt	1991-1993	20	n.r.	n.r.	n.r.	100	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	El-Sayed, 1996

n.a.: not applicable; n.r. not reported; LC: left censored data (values below the LOD or LOQ); LOD: Limit of detection; LOQ: Limit of quantification; Min: minimum; Max: maximum; P50: 50th percentile; UK: United Kingdom; USA: United States of America; ELISA: Enzyme linked immunosorbent assay; HPLC: High-performance liquid chromatography; TLC: Thin-layer chromatography; LC-MS: Liquid chromatography mass spectrometry; CIT: Citrinin; OTA: ochratoxin A; AFB1: Aflatoxin B_1, AFB2: Aflatoxin B_2; AF: Aflatoxin; PAT: Patulin.
(a): Year of sample collection.
(b): not premium grade fruits, but fruits that showed characteristic brown discoloration.
(c): number of samples (% of samples) in which the mentioned mycotoxins occurred together.
(d): µg/L.
(e): descriptive statistics of numerical data (above LOD or LOQ) as reported by the authors.

Table B3: Overview of previously reported literature data on occurrence of citrinin in feed.

Commodity	Origin	Year [(a)]	n	LOD (µg/kg)	LOQ (µg/kg)	Analytical method	% LC	Descriptive statistics (µg/kg)[(i)]				Reported levels (µg/kg)	Co-occurrence with OTA, AF, PAT[(d)]	Reference
								Min	P50	Max	Mean			
Europe														
Feed	Romania	2007	55	n.r.	n.r.	ELISA	0	17	n.r.	> 405	n.r.	< 101: 41.8%[(c)] 101-200:21.8% 201-400: 10.9% > 405: 25.5%	n.r.	Talmaciu et al., 2008
Feed (pig/chicken)	Bulgaria	2006	25	n.r.	n.r.	HPLC	8	n.r.	n.r.	n.r.	54.7 ± 27.5[(f)]	n.r.	Co-occurrence with OTA	Stoev et al., 2010
Feed (pig/chicken)	Bulgaria	2007	25	n.r.	n.r.	HPLC	4	n.r.	n.r.	n.r.	120.5 ± 43.3[(f)]	n.r.	Co-occurrence with OTA	Stoev et al., 2010
Mature maize silage (11 months old)	France	2006	2 times 8	1.5	5	LC-MS	0	n.r.	n.r.	n.r.	11.2 ± 0.73 (top) 12.5 ± 1.3 (bottom) [(g, h)]	n.r.	CIT+AFB1: 2 (100 %)	Richard et al., 2009
Mature maize silage (11 months old)	France	n.r.	2 times 8	n.r.	n.r.	LC-MS	0	n.r.	n.r.	n.r.	26.9 ± 2.5 (top) 36.6 ± 2.3 (bottom) [(g, h)]	n.r.	No co-occurrence with AFB1 or OTA	Richard et al., 2007
Maize silage	France	2004	9[(b)]	1.5	5	LC-MS	0	5	n.r.	25	n.r.	n.r.	AFB1+CIT: 9 (100 %)	Garon et al., 2006
Compounded feeds	Russia	2003 - 2006	829	10	n.r.	ELISA	91	12	n.r.	182	n.r.	n.r.	n.r.	Kononenko and Burkin, 2008
Soy-bean oilseed meal and cakes	Russia	2003 - 2006	148	10	n.r.	ELISA	98	14	20	30	n.r.	14; 20; 30	n.r.	Kononenko and Burkin, 2008
Sunflower oilseed meal and cakes	Russia	2003 - 2006	142	10	n.r.	ELISA	71	14	n.r.	397	n.r.	n.r.	n.r.	Kononenko and Burkin, 2008
Wheat bran	Russia	2003 - 2006	37	10	n.r.	ELISA	97	n.a.	n.a.	n.a.	n.a.	50	n.r.	Kononenko and Burkin, 2008
Maize gluten	Russia	2003 - 2006	49	10	n.r.	ELISA	84	16	n.r.	62	n.r.	n.r.	n.r.	Kononenko and Burkin, 2008
Wheat bran (feed)	Bulgaria	1998	24	5	n.r.	ELISA	79	5.9	10	230	n.r.	5.9; 6.1; 10; 36; 230[(e)]	CIT + OTA: 5	Vrabcheva et al., 2000
Rice bran (feed ingredient)	UK	1992	40	20	n.r.	HPLC	100	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	Scudamore et al., 1997

Table B3: Continued.

Commodity	Origin	Year [(a)]	n	LOD (µg/kg)	LOQ (µg/kg)	Analytical method	% LC	Descriptive statistics (µg/kg) [(i)]				Reported levels (µg/kg)	Co-occurrence with OTA, AF, PAT[(d)]	Reference
								Min	P50	Max	Mean			
Maize gluten and products (feed ingredient)	UK	1992	50	20	n.r.	HPLC	100	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	Scudamore et al., 1997
Cottonseed meal (feed ingredient)	UK	1992	21	20	n.r.	HPLC	100	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	Scudamore et al., 1997
Rapeseed (feed ingredient)	UK	1992	25	5	n.r.	HPLC	100	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	Scudamore et al., 1997
Sunflower meal (feed ingredient)	UK	1992	20	10	n.r.	HPLC	100	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	Scudamore et al., 1997
Olive pulp (feed ingredient)	UK	1992	5	5	n.r.	HPLC	100	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	Scudamore et al., 1997
Palm products (feed ingredient)	UK	1992	15	5	n.r.	HPLC	93	n.a.	n.a.	n.a.	n.a.	7	AF+OTA+CIT: 1 (7 %)	Scudamore et al., 1997
Soya (feed ingredient)	UK	1992	20	5	n.r.	HPLC	100	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	Scudamore et al., 1997
Peas/beans (feed ingredient)	UK	1992	15	5	n.r.	HPLC	93	n.a.	n.a.	n.a.	n.a.	9	OTA+CIT: 1 (7 %)	Scudamore et al., 1997
Manioc (feed ingredient)	UK	1992	10	5	n.r.	HPLC	100	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	Scudamore et al., 1997
Citrus pulp (feed ingredient)	UK	1992	14	5	n.r.	HPLC	100	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	Scudamore et al., 1997

n.a.: not applicable; n.r. not reported; LC: left censored data (values below the LOD or LOQ); LOD: Limit of detection; LOQ: Limit of quantification; Min: minimum; Max: maximum; P50: 50th percentile; UK: United Kingdom; ELISA: Enzyme linked immunosorbent assay; HPLC: High-performance liquid chromatography; LC-MS: Liquid chromatography mass spectrometry; CIT: citrinin; OTA: ochratoxin A; AFB1: Aflatoxin B_1; AF: Aflatoxin

(a): Year of sample collection.

(b): 6 Samples were taken every month (during 9 months) and reported as mean ± standard deviation per month.

(c): All reported values are given in the paper.

(d): number of samples (% of samples) in which the mentioned mycotoxins occurred together.

(e): reported values are not corrected for recovery.

(f): mean ± standard error of the mean.

(g): expressed as µg/kg dry matter.

(h): mean ± standard deviation.

(i): descriptive statistics of numerical data (above LOD or LOQ) as reported by the authors.

C. CONSUMPTION DATA AND CRITICAL CITRININ CONCENTRATIONS

Table C1: Critical citrinin concentration in grains and grain-based products that may cause a chronic dietary exposure equal to the level of no concern for nephrotoxicity in infants.

INFANTS				Consumption amount (g/kg b.w. per day)[(b)]		Citrinin concentration (µg/kg) of concern for average and P95 consumers[(c)]	
Country	**Survey**	**Number of subjects**	**% consumers[(a)]**	**Average**	**P95**	**Average**	**P95**
Bulgaria	NUTRICHILD	860	53.3	3.1	12.1	64.5	16.5
Italy	INRAN_SCAI_2005_06	16	31.3	1.8	12.7[(d)]	111.1	-

b.w.: body weight; P95: 95th percentile.

(a): Percentage of subjects reporting consumption of grains and grain-based products in the dietary survey.

(b): Average and P95 consumption of grains and grain-based products (FoodEx level 1) as retrieved from the Comprehensive database. Data available from http://www.efsa.europa.eu/en/datex/datexfooddb.htm (Chronic food consumption statistics reported in g/kg b.w. per day).

(c): The critical citrinin concentration has been derived by dividing the level of no concern for nephrotoxicity (0.2 µg/kg/bw) by the consumption amount (g/kg b.w. per day) of average and high percentile (P95) consumption of grains and grain-based products.

(d): P95 estimates for dietary surveys/age classes with less than 60 observations may not be statistically robust (EFSA, 2011b) and therefore they should not be considered in the risk characterisation.

Table C2: Critical citrinin concentration in grains and grain-based products that may cause a chronic dietary exposure equal to the level of no concern for nephrotoxicity in toddlers.

TODDLERS				Consumption amount (g/kg b.w. per day) for consumers only[(b)]		Citrinin concentration (µg/kg) of concern for average and P95 consumers[(c)]	
Country	**Survey**	**Number of subjects**	**% consumers[(a)]**	**Average**	**P95**	**Average**	**P95**
Belgium	FPDS_1	36	100.0	9.3	15.3[(d)]	21.5	-
Bulgaria	NUTRICHILD	428	99.5	10.5	17.4	19.0	11.5
Finland	DIPP	497	100.0	5.9	11.8	33.9	16.9
Germany	DONALD_2006	92	98.9	7	12.9	28.6	15.5
Germany	DONALD_2007	85	97.6	6.9	12.3	29.0	16.3
Germany	DONALD_2008	84	98.8	6.6	12.1	30.3	16.5
Italy	INRAN_SCAI_2005_06	36	97.2	9.5	18.8[(d)]	21.1	-
Netherlands	VCP_kids	322	100.0	8.4	15.2	23.8	13.2
Spain	enKid	17	100.0	5.9	10.5[(d)]	33.9	-
			Minimum	**5.9**	**10.5**	**19.0**	**11.5**
			Median	**7.0**	**12.9**	**28.6**	**15.9**
			Maximum	**10.5**	**18.8**	**33.9**	**16.9**

b.w.: body weight; P95: 95th percentile.

(a): Percentage of subjects reporting consumption of grains and grain-based products in the dietary survey.

(b): Average and P95 consumption of grains and grain-based products (FoodEx level 1) as retrieved from the Comprehensive database. Data available from http://www.efsa.europa.eu/en/datex/datexfooddb.htm (Chronic food consumption statistics reported in g/kg b.w. per day).

(c): The citrinin concentration of concern has been derived by dividing the level of no concern for nephrotoxicity (0.2 µg/kg b.w. per day) by the consumption amount (g/kg b.w. per day) of average and high percentile (P95) consumption of grains and grain-based products.

(d): P95 estimates for dietary surveys/age classes with less than 60 observations may not be statistically robust (EFSA, 2011b) and therefore they should not be considered in the risk characterisation.

Table C3: Critical citrinin concentration in grains and grain-based products that may cause a chronic dietary exposure equal to the level of no concern for nephrotoxicity in 'other children'.

OTHER CHILDREN				Consumption amount (g/kg b.w. per day) for consumers only[(b)]		Citrinin concentration (µg/kg) of concern for average and P95 consumers[(c)]	
Country	**Survey**	**Number of subjects**	**% consumers[(a)]**	**Average**	**P95**	**Average**	**P95**
Belgium	FPDS_1	625	100.0	7.9	14	25.3	14.3
Bulgaria	NUTRICHILD	433	99.8	10.6	19.6	18.9	10.2
Czech Republic	SISP04	389	100.0	7.9	15.1	25.3	13.2
Denmark	Danish_Dietary_Survey	490	100.0	7.9	12.6	25.3	15.9
Finland	DIPP	933	100.0	5.1	8.6	39.2	23.3
Finland	STRIP	250	100.0	8.1	14	24.7	14.3
France	INCA2	482	100.0	7.8	13.6	25.6	14.7
Germany	DONALD_2006	211	100.0	6.8	11.2	29.4	17.9
Germany	DONALD_2007	226	100.0	6.8	11.1	29.4	18.0
Germany	DONALD_2008	223	100.0	7.1	11.1	28.2	18.0
Greece	Regional_Crete	839	99.5	5.9	11.2	33.9	17.9
Italy	INRAN_SCAI_2005_06	193	100.0	9.3	17.1	21.5	11.7
Latvia	EFSA_TEST	189	100.0	6	14	33.3	14.3
Netherlands	VCP_kids	957	100.0	7.4	13.1	27.0	15.3
Spain	NUT_INK05	156	100.0	6.7	11.9	29.9	16.8
Spain	enKid	399	100.0	7.4	13.1	27.0	15.3
Sweden	Riksmaten_barn	1 473	100.0	9.2	23.3	21.7	8.6
			Minimum	**5.1**	**8.6**	**18.9**	**8.6**
			Median	**7.4**	**13.1**	**27.0**	**15.3**
			Maximum	**10.6**	**23.3**	**39.2**	**23.3**

b.w.: body weight; P95: 95th percentile.

(a): Percentage of subjects reporting consumption of grains and grain-based products in the dietary survey.

(b): Average and P95 consumption of grains and grain-based products (FoodEx level 1) as retrieved from the Comprehensive database. Data available from http://www.efsa.europa.eu/en/datex/datexfooddb.htm (Chronic food consumption statistics reported in g/kg b.w. per day).

(c): The citrinin concentration of concern has been derived by dividing the level of no concern for nephrotoxicity (0.2 µg/kg b.w. per day) by the consumption amount (g/kg b.w. per day) of average and high percentile (P95) consumption of grains and grain-based products.

Table C4: Critical citrinin concentration in grains and grain-based products that may cause a chronic dietary exposure equal to the level of no concern for nephrotoxicity in adolescents.

ADOLESCENTS				Consumption amount (g/kgb.w. per day) for consumers only[(b)]		Citrinin concentration (µg/kg) of concern for average and P95 consumers[(c)]	
Country	**Survey**	**Number of subjects**	**% consumers[(a)]**	**Average**	**P95**	**Average**	**P95**
Belgium	Diet_National_2004	584	100.0	4.3	8.1	46.5	24.7
Cyprus	Childhealth	303	99.7	3.2	6.2	62.5	32.3
Czech Republic	SISP04	298	100.0	6.1	12	32.8	16.7
Denmark	Danish_Dietary_Survey	479	100.0	4.3	7.6	46.5	26.3
France	INCA2	973	100.0	5	9.2	40.0	21.7
Germany	National_Nutrition_Survey_II	1 011	99.8	3.7	7.6	54.1	26.3
Italy	INRAN_SCAI_2005_06	247	100.0	5.5	10.4	36.4	19.2
Latvia	EFSA_TEST	470	99.6	4.3	9.3	46.5	21.5
Spain	AESAN_FIAB	86	100.0	3.5	6.3	57.1	31.7
Spain	NUT_INK05	209	100.0	5	9.9	40.0	20.2
Spain	enKid	651	100.0	5	9.1	40.0	22.0
Sweden	Riksmaten_barn	1 018	99.9	4.9	9.5	40.8	21.1
			Minimum	**3.2**	**6.2**	**32.8**	**16.7**
			Median	**4.6**	**9.2**	**43.7**	**21.9**
			Maximum	**6.1**	**12.0**	**62.5**	**32.3**

b.w.: body weight; P95: 95th percentile.

(a): Percentage of subjects reporting consumption of grains and grain-based products in the dietary survey.

(b): Average and P95 consumption of grains and grain-based products (FoodEx level 1) as retrieved from the Comprehensive database. Data available from http://www.efsa.europa.eu/en/datex/datexfooddb.htm (Chronic food consumption statistics reported in g/kg b.w. per day).

(c): The citrinin concentration of concern has been derived by dividing the level of no concern for nephrotoxicity (0.2 µg/kg b.w. per day) by the consumption amount (g/kg b.w. per day) of average and high percentile (P95) consumption of grains and grain-based products.

Table C5: Critical citrinin concentration in grains and grain-based products that may cause a chronic dietary exposure equal to the level of no concern for nephrotoxicity in adults.

ADULTS				Consumption amount (g/kgb.w. per day) for consumers only[(b)]		Citrinin concentration (µg/kg) of concern for average and P95 consumers[(c)]	
Country	**Survey**	**Number of subjects**	**% consumers[(a)]**	**Average**	**P95**	**Average**	**P95**
Belgium	Diet_National_2004	1 304	99.6	3.4	6.7	58.8	29.9
Czech Republic	SISP04	1 666	99.9	3.7	7	54.1	28.6
Denmark	Danish_Dietary_Survey	2 822	100.0	3	5.1	66.7	39.2
Finland	FINDIET_2007	1 575	99.9	2	3.8	100.0	52.6
France	INCA2	2 276	100.0	3.4	6.1	58.8	32.8
Germany	National_Nutrition_Survey_II	10 419	99.8	3	6	66.7	33.3
Hungary	National_Repr_Surv	1 074	100.0	3.4	5.9	58.8	33.9
Ireland	NSIFCS	958	100.0	3	5.4	66.7	37.0
Italy	INRAN_SCAI_2005_06	2 313	100.0	3.7	6.4	54.1	31.3
Latvia	EFSA_TEST	1 306	99.3	3	6.7	66.7	29.9
Netherlands	DNFCS_2003	750	99.9	3.1	5.5	64.5	36.4
Spain	AESAN	410	100.0	2.6	5.2	76.9	38.5
Spain	AESAN_FIAB	981	99.9	2.7	5.2	74.1	38.5
Sweden	Riksmaten_1997_98	1 210	100.0	3.2	6.3	62.5	31.7
United Kingdom	NDNS	1 724	99.8	3.1	5.6	64.5	35.7
			Minimum	**2.0**	**3.8**	**54.1**	**28.6**
			Median	**3.1**	**5.9**	**64.5**	**33.9**
			Maximum	**3.7**	**7.0**	**100.0**	**52.6**

b.w.: body weight; P95: 95th percentile.

(a): Percentage of subjects reporting consumption of grains and grain-based products in the dietary survey.

(b): Average and 95 percentile consumption of grains and grain-based products (FoodEx level 1) as retrieved from the Comprehensive database. Data available from http://www.efsa.europa.eu/en/datex/datexfooddb.htm (Chronic food consumption statistics reported in grams/kg b.w. per day).

(c): The citrinin concentration of concern has been derived by dividing the level of no concern for nephrotoxicity (0.2 µg/kg b.w. per day) by the consumption amount (g/kg b.w. per day) of average and high percentile (P95) consumption of grains and grain-based products.

Table C6: Critical citrinin concentration in grains and grain-based products that may cause a chronic dietary exposure equal to the level of no concern for nephrotoxicity in elderly.

ELDERLY				Consumption amount (g/kgb.w. per day) for consumers only[(b)]		Citrinin concentration (µg/kg) of concern for average and P95 consumers[(c)]	
Country	**Survey**	**Number of subjects**	**% consumers[(a)]**	**Average**	**P95**	**Average**	**P95**
Belgium	Diet_National_2004	518	99.8	2.7	5.8	74.1	34.5
Denmark	Danish_Dietary_Survey	309	100.0	2.7	4.6	74.1	43.5
Finland	FINDIET_2007	463	100.0	2	3.7	100.0	54.1
France	INCA2	264	99.6	3.1	6	64.5	33.3
Germany	National_Nutrition_Survey_II	2 006	99.8	3	5.6	66.7	35.7
Hungary	National_Repr_Surv	206	100.0	3.2	5	62.5	40.0
Italy	INRAN_SCAI_2005_06	290	100.0	3.4	6	58.8	33.3
			Minimum	2.0	3.7	58.8	33.3
			Median	3.0	5.6	66.7	35.7
			Maximum	3.4	6.0	100.0	54.1

b.w.: body weight; P95: 95th percentile.

(a): Percentage of subjects reporting consumption of grains and grain-based products in the dietary survey.

(b): Average and P95 consumption of grains and grain-based products (FoodEx level 1) as retrieved from the Comprehensive database. Data available from http://www.efsa.europa.eu/en/datex/datexfooddb.htm (Chronic food consumption statistics reported in g/kg b.w. per day).

(c): The citrinin concentration of concern has been derived by dividing the level of no concern for nephrotoxicity (0.2 µg/kg b.w. per day) by the consumption amount (g/kg b.w. per day) of average and high percentile (P95) consumption of grains and grain-based products.

Table C7: Critical citrinin concentration in grains and grain-based products that may cause a chronic dietary exposure equal to the level of no concern for nephrotoxicity in very elderly

VERY ELDERLY				Consumption amount (g/kgb.w. per day) for consumers only[(b)]		Citrinin concentration (µg/kg) of concern for average and P95 consumers[(c)]	
Country	**Survey**	**Number of subjects**	**% consumers[(a)]**	**Average**	**P95**	**Average**	**P95**
Belgium	Diet_National_2004	712	99.7	2.6	5.3	76.9	37.7
Denmark	Danish_Dietary_Survey	20	100.0	2.8	4.9[(d)]	71.4	-
France	INCA2	84	100.0	2.8	5.5	71.4	36.4
Germany	National_Nutrition_Survey_II	490	100.0	3	6.5	66.7	30.8
Hungary	National_Repr_Surv	80	100.0	3.5	5.8	57.1	34.5
Italy	INRAN_SCAI_2005_06	228	100.0	3.7	6.4	54.1	31.3
			Minimum	**2.6**	**5.3**	**54.1**	**30.8**
			Median	**2.9**	**5.8**	**69.0**	**34.5**
			Maximum	**3.7**	**6.5**	**76.9**	**37.7**

b.w.: body weight; P95: 95th percentile.

(a): Percentage of subjects reporting consumption of grains and grain-based products in the dietary survey.

(b): Average and 95 percentile consumption of grains and grain-based products (FoodEx level 1) as retrieved from the Comprehensive database. Data available from http://www.efsa.europa.eu/en/datex/datexfooddb.htm (Chronic food consumption statistics reported in g/kg b.w. per day).

(c): The citrinin concentration of concern has been derived by dividing the level of no concern for nephrotoxicity (0.2 µg/kg b.w. per day) by the consumption amount (g/kg b.w. per day) of average and high percentile (P95) consumption of grains and grain-based products.

(d): P95 estimates for dietary surveys/age classes with less than 60 observations may not be statistically robust (EFSA, 2011b) and therefore they should not be considered in the risk characterisation.

ABBREVIATIONS

AESAN	Spanish Food and Drink Industry Federation
AESAN_FIAB	Spanish Food and Drink Industry Federation – Spanish dietary survey
AF	Aflatoxin
AFB1,2	Aflatoxin $B_{1,2}$
ATP	Adenosine triphosphate
BEN	Balkan endemic nephropathy
b.w.	Body weight
Childheath	Childhealth (Cyprus, Dietary survey)
CHO	Chinese hamster ovary
CIT	Citrinin
CONTAM Panel	EFSA Panel on Contaminants in the Food Chain
Danish_Dietary_Survey	Danish dietary survey (Denmark, Dietary Survey)
DCM	EFSA Dietary and Chemical Monitoring Unit (former DATEX Unit)
Diet_National_2004	Diet_National_2004 (Belgium, Dietary survey)
DIPP	DIPP (Finland, Dietary survey)
DNFCS_2003	Dutch National Food Consumption Survey
DONALD_2006	DONALD_2006 (Germany, Dietary survey)
DONALD_2007	DONALD_2007 (Germany, Dietary survey)
DONALD_2008	DONALD_2008 (Germany, Dietary survey)
EFSA	European Food Safety Authority
EFSA-TEST	EFSA_TEST (Latvia, Dietary survey)
ELISA	Enzyme linked immunosorbent assay
enKid	Food preferences of Spanish children and young people (Spain, Dietary survey)
ERK	Extracellular-signal-regulated kinase
ESC	Embryonic stem cells
ESI	Electrospray ionization
EU	European Union
EXPOCHI	EFSA Article 36 project 'Individual food consumption data and exposure assessment studies for children'
FAO	Food and Agriculture Organization
FINDIET_2007	FINDIET 2007 (Finland, Dietary survey)
FLD	Fluorescence detection
FPDS_1	FPDS_1 (Belgium, Dietary survey)
FPG	Formamidopyrimidine-DNA glycosylase
GC-MS	Gas chromatography mass spectrometry
GSH	Glutathione
HEK	Human embryonic kidney
HK	Human kidney
HL-60	Human promyelocitic leukemia
HPLC	High-performance liquid chromatography
hOGG1	Human 8-hydroxyguanine DNA glycosylase 1
HSP	Heat shock protein
IAC	Immunoaffinity columns
IFN	Interferon
IL	Interleukin
INCA2	Enquête Individuelle et Nationale sur les Consommations Alimentaires (France, Dietary survey)
INRAN_SCAI_2005_06	INRAN_SCAI_2005_06 (Italy, Dietary survey)
i.p.	Intraperitoneal
IUPAC	international union of pure and applied chemistry
JUN	c-Jun N-terminal kinase
LC	Left censored

LC-MS	Liquid chromatography mass spectrometry
LOAEL	Lowest-observed-adverse-effect level
LOD	Limit of detection
LOQ	Limit of quantification
LPS	Lipopolysaccharide
MDA	Malondialdehyde
MIP	Molecularly imprinted polymer
MLs	Maximum levels
MN	Micronuclei
MS/MS	Tandem mass spectrometry
N	Number of samples
National_Nutrition_Survey_II	National_Nutrition_Survey_II (Germany, Dietary survey)
National_Repr_Surv	National_Repr_Surv (Hungary, Dietary survey)
NDMA	N-nitrosodimethylamine
NDNS	National Diet and Nutrition Survey (United Kingdom, Dietary survey)
NMR	Nuclear magnetic resonance
NO	Nitric oxide
NOAEL	No-observed-adverse-effect level
NPB	nucleoplasmic bridges
n.r.	Not reported
NSIFCS	North/South Ireland Food Consumption Survey (Ireland, Dietary survey)
NUT-INK05	NUT-INK05 (Spain, Dietary survey)
NUTRICHILD	NUTRICHILD (Bulgaria, Dietary survey)
OTA	Ochratoxin A
PAK2	p21-activated protein kinase 2
PAT	Patulin
PCT	Proximal convoluted tubules
PDA	Photodiode-array
PMBC	Peripheral blood mononuclear cells
QuEChERS	Quick Easy Cheap Effective Rugged Safe
Regional Crete	Regional Crete (Greece, Dietary survey)
Riksmaten_1997_98	Riksmaten_1997_98 (Sweden, Dietary Survey)
Riksmaten_barn	Riksmaten_barn (Sweden, Dietary Survey
RMR	Red mould rice
ROS	Reactive oxygen species
RP	Reversed phase
s.c.	Subcutaneous
SCE	Sister chromatid exchange
SCGE	Single cell gell electrophoresis
SISP04	SISP04 (Czech Republic, Dietary survey)
SRBC	Sheep red blood cells
STRIP	STRIP (Finland, Dietary survey)
RT-PCR	reverse transcription polymerase chain reaction
SIM	Selected ion monitoring
SPE	Solid phase extraction
TLC	Thin-layer chromatography
SRBC	Sheep red blood cells
UK	The United Kingdom
USA	The United States of America
UV	Ultraviolet
VCP_kids	VCP_kids (The Netherlands, Dietary survey)
WHO	World Health Organization