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Cyclopiazonic acid: 50th anniversary of its discovery

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REVIEW ARTICLE

Abstract

In 1968, the mycotoxin cyclopiazonic acid (CPA) was first discovered and characterised as a chemical substance. Within the following five decades, much has been learned from the results of CPA research. CPA is produced by several *Penicillium* species (*P. griseofulvum*, *P. camemberti, P. commune*, *P. dipodomyicola*) and *Aspergillus* species (*A. flavus, A. oryzae* and *A. tamarii*). It is widespread on naturally contaminated agricultural raw materials. CPA has been reported to occur in food commodities (e.g. oilseeds, nuts, cereals, dried figs, milk, cheese and meat products) and to possess toxicological significance. CPA is also frequently detected in peanuts and maize; the presence of CPA and aflatoxins in maize and peanuts contaminated with *A. flavus* suggests that synergism may occur. CPA is toxic to several animal species, such as rats, pigs, guinea pigs, poultry and dogs. After ingesting CPA-contaminated feeds, test animals display severe gastrointestinal upsets and neurological disorders. Organs affected include the liver, kidney, heart, and digestive tract, which show degenerative changes and necrosis. Biologically, CPA is a specific inhibitor of sarco(endo)plasmic reticulum Ca^{2+}-ATPase. Data from toxicological evaluation of aflatoxins and CPA in broiler chickens demonstrate that both aflatoxins and CPA alone and the aflatoxin-CPA combination can adversely affect broiler health. The effects of aflatoxins and CPA combination were additive in most cases.

Keywords: cyclopiazonic acid, anniversary, producers, food, health

1. Introduction

Cyclopiazonic acid (CPA) was originally discovered and chemically characterised by Holzapfel (1968). For the remainder of this review, the term CPA refers to α-CPA. CPA was isolated from culture extracts as a main toxic metabolite of *Penicillium cyclopium* Westling (strain CSIR 1082) (correct name: *Penicillium griseofulvum* Dierckx) during routine toxicity screening of microfungi. *P. cyclopium* was isolated from groundnuts that caused acute toxicosis in ducklings and rats (Holzapfel, 1968).

In a later study, Holzapfel *et al.* (1970) reported two new relatively non-toxic indole derivatives: bissecodehydro cyclopiazonic acid (β-CPA) and α-cyclopiazonic acid-imine (α-CPA-imine), which were also produced by *P. cyclopium*. Subsequent studies on the two intermediates revealed that they were relatively non-toxic compared to CPA. β-CPA was found to be a direct precursor of CPA (De Jesus *et al.*, 1981; Holzapfel and Wilkins, 1971; Steyn *et al.*, 1975). The above-mentioned metabolites, namely β-CPA and α-CPA-imine, are derivatives of CPA which belong to the CPA-type mycotoxins of the indole subclass. Since 1970, approximately 27 CPA-type mycotoxins have been reported in different fungal extracts of *Aspergillus* and *Penicillium* species, e.g. CPA-type mycotoxins:indole derivatives (β-CPA, α-CPA-imine, iso-α-CPA, cyclo-acetoacetyl-L-tryptophan (cAATrp), pseuboydone E) and CPA-type mycotoxins:oxindole derivatives (2-oxo CPA, speradines, aspergillines and cyclopiamides) (Hu *et al.*, 2014; Ma *et al.*, 2015; Uka *et al.*, 2017).

CPA is an indole tetramic and a lipophilic monobasic acid that has a structural resemblance to lysergic acid (Holzapfel and Wilkins, 1971). CPA possesses a metal-chelating ability. It was demonstrated that the previously isolated flavutoxin

(sodium cyclopiazonate) is the metal chelate-complex of CPA (Gallagher *et al.*, 1978; Kirksey and Cole, 1973).

The potential toxic risk of CPA to humans and animals was initially considered as low because it was less potent compared with other secondary metabolites of toxigenic microfungi (e.g. aflatoxin and sterigmatocystin) (Purchase, 1971). Consequently, CPA did not attract the attention of the scientific community. Scientific interest in CPA increased five years later: in 1977, it was demonstrated that a prolific and important food and feed contaminant *Aspergillus flavus* could produce CPA. Gradually, CPA research focused on the co-occurrence of CPA and aflatoxin in the food and feed chain (Dorner *et al.*, 1983, 1984; Gallagher *et al.*, 1978; Luk *et al.*, 1977; Morrissey *et al.*, 1987).

There is very little proof for human toxicity due to consumption of food contaminated by CPA. Nonetheless, the isolation of CPA from two batches of kodo millet (*Paspalum scrobiculatum*) grain associated with incidents of 'kodua poisoning' in humans and cattle in India has been reported by Rao and Husain (1985). Furthermore, it was demonstrated that strains of *A. flavus* and *Aspergillus tamarii* detected in this contaminated millet produced CPA. Unfortunately, CPA concentration was not determined in the affected millet.

The economic significance of CPA as a food contaminant was enhanced when Still *et al.* (1978) and Le Bars (1979a) found isolates of *Penicillium camemberti* Thom that produced CPA; CPA was produced in *P. camemberti* cultures and in cheeses stored under unusual conditions (five days at 25 °C) with concentrations as high as 4 mg/kg. CPA has been reported historically also to occur in food commodities of plant origin. CPA is frequently detected in peanuts and maize (Lansden, 1986; Lansden and Davidson, 1983; Reddy and Reddy, 1992; Urano *et al.*, 1992a,b; Widiastuti, *et al.*, 1988).

This article is a review of the informative data published on CPA research since its discovery fifty years ago, using the systematic literature review methodology. The preparation of this review and verification of the published information was indeed a 'detective investigation'. Some of the identified old CPA reprints came from our archive, and some others were unfortunately no longer available. The principal milestones in CPA discovery and research in 1968-1995 and 1996-2017 are summarised in Figure 1 and 2.

2. Cyclopiazonic acid chemistry

The chemical structure of CPA and its chemical data are shown in Figure 3. The chemical structures of CPA-type mycotoxins: the indole and oxindole derivatives (speradines, aspergillines, cyclopiamides) are presented in Figure 4-7 (Uka *et al.*, 2017).

Chemical and physical properties of CPA were comprehensively described by Holzapfel (1971), Cole and Cox (1981) and Cole (1984). CPA is a tetramic indole acid. It is produced from the mevalonate pathway, tryptophan and two acetate molecules. CPA is an optically active, colourless, odourless, crystalline metabolite. For analytical purposes, CPA is soluble in chloroform, dichloromethane, methanol and acetonitrile, and sodium bicarbonate. The solubility of CPA in these solvents is approximately 20 mg/ml. CPA is insoluble in water and sparingly soluble in aqueous buffers. The following information are significant for the conduction of toxicological and bioavailability studies. CPA is soluble in dimethyl sulfoxide (DMSO) (33.64 mg/ml), DMSO:phosphate-buffered saline (PBS) (1:1, pH 7.2) (0.5 mg/ml), ethanol (1.68 mg/ml) and dimethylformamide (20 mg/ml). CPA is stable when stored in dry state at 4 °C (Cole, 1984; Cole and Cox, 1981; Holzapfel, 1971). Diaz *et al.* (2010) reported on the stability of CPA in solution. CPA is unstable in the presence of formic acid and oxygen, and readily adsorbs to polypropylene. Standard solutions intended for liquid chromatography analysis should be prepared in glass vials, in water and organic solvent, with no acid added. In order to prevent deterioration of the CPA standard from exposure to heat and ambient or headspace air, ascorbic acid can be added to the standard solution and the vials containing the standard should be filled to the top. Since ascorbic acid significantly reduces CPA loss, it may be possible that ascorbic acid added to samples could reduce losses of CPA during extraction processes. Due to its low stability in DMSO:PBS (1:1, pH 7.2), it is not recommended to store CPA for more than one day in this solution. Maragos (2009) showed that CPA, which is non-fluorescent, can be rendered fluorescent upon photolysis.

Although the structure of CPA has been known since 1968, only three racemic syntheses have been published to date (Haskins and Knight, 2005; Kozikowski *et al.*, 1984; Muratake and Natsume, 1985) and very little is known on the related structure-activity relationships. Beyer *et al.* (2010, 2011) reported the first structure-activity data of several CPA derivatives and stereoisomers as well as the first asymmetric total synthesis of CPA by a modification of the Knight synthesis, currently the most efficient route to produce CPA.

3. Cyclopiazonic acid producers in foodstuffs

CPA is produced by many ascomycetous microfungi in genera *Penicillium* and *Aspergillus*. *P. cyclopium* Westling strain CSIR 1082 was the first producer of CPA ever identified (Holzapfel, 1968). This microfungus was originally described as *Penicillium urticae* strain G391, which was isolated from groundnuts (Scott, 1965). After a few years, it was reclassified as *P. cyclopium* (Purchase, 1971), and later as *P. griseofulvum* Dierckx (Reddy and Reddy, 1987).

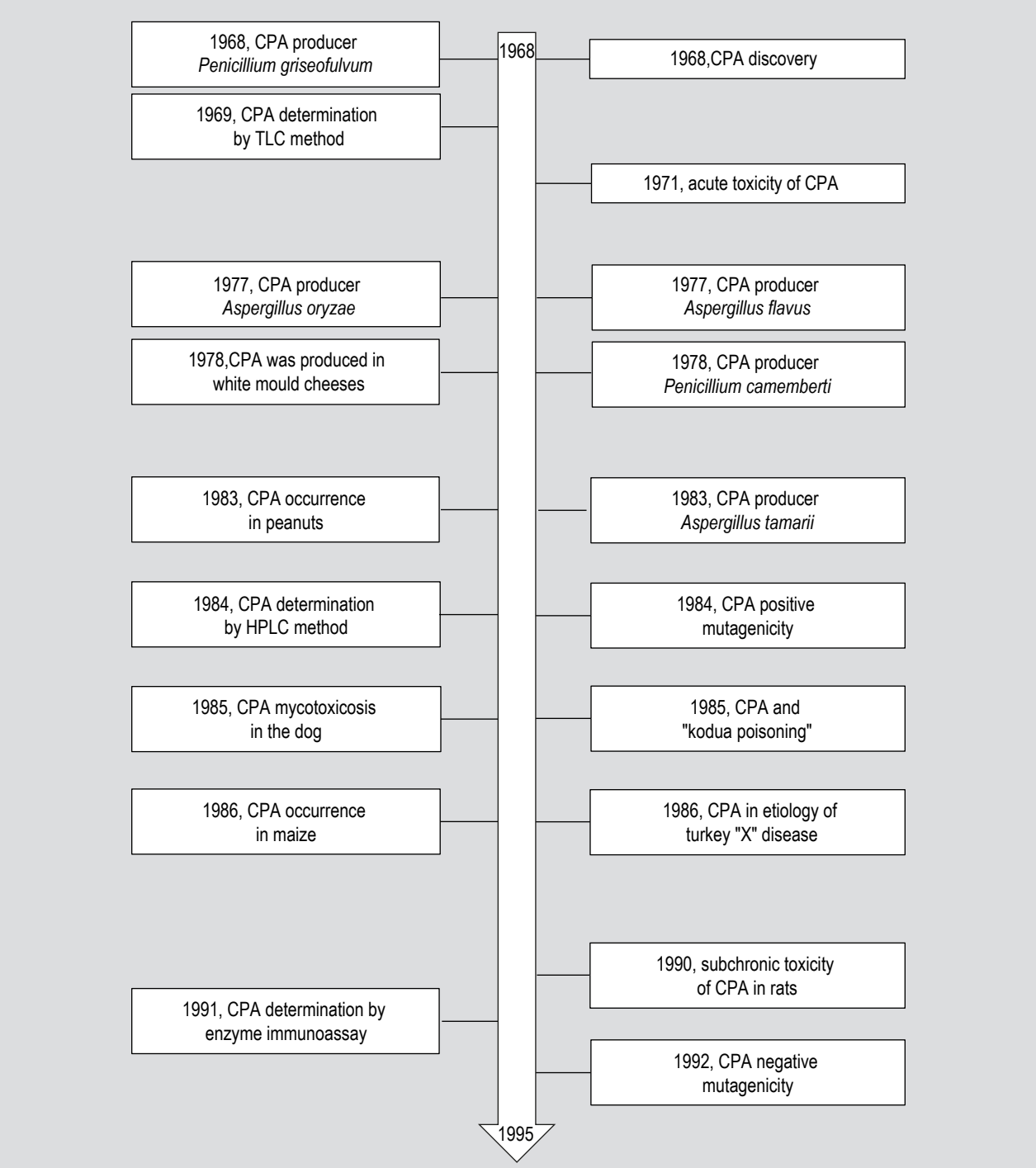


Figure 1. The principal milestones in cyclopiazonic acid (CPA) research in 1968-1995.

It was also reported that CPA could be produced by a strain of *Aspergillus versicolor* (Vuill.) Tirab. (Ohmomo *et al.*, 1973). Though it had been very often cited as such, it was eventually concluded that *A. versicolor* is not a producer of CPA (Domsch *et al.*, 1980). The original culture classified as *A. versicolor* was actually mistaken with *Aspergillus oryzae* (Frisvad, 1989; Orth, 1977).

In 1977, it was reported that *A. flavus* could produce CPA (Luk *et al.*, 1977), and this production was confirmed subsequently (Gallagher *et al.*, 1978; Georgianna *et al.*, 2010; Ostry, 1989; Polster *et al.*, 1990). Since then, other fungal species, such as *P. camemberti* Thom (Geisen *et al.*, 1990; Le Bars, 1979a,b; Ostry, 1989; Ostry *et al.*, 1991; Pitt *et al.*, 1986; Still *et al.*, 1978), *Penicillium commune* Thom (Leistner and Pitt, 1977; Pitt *et al.*, 1986; Sosa *et al.*, 2002),

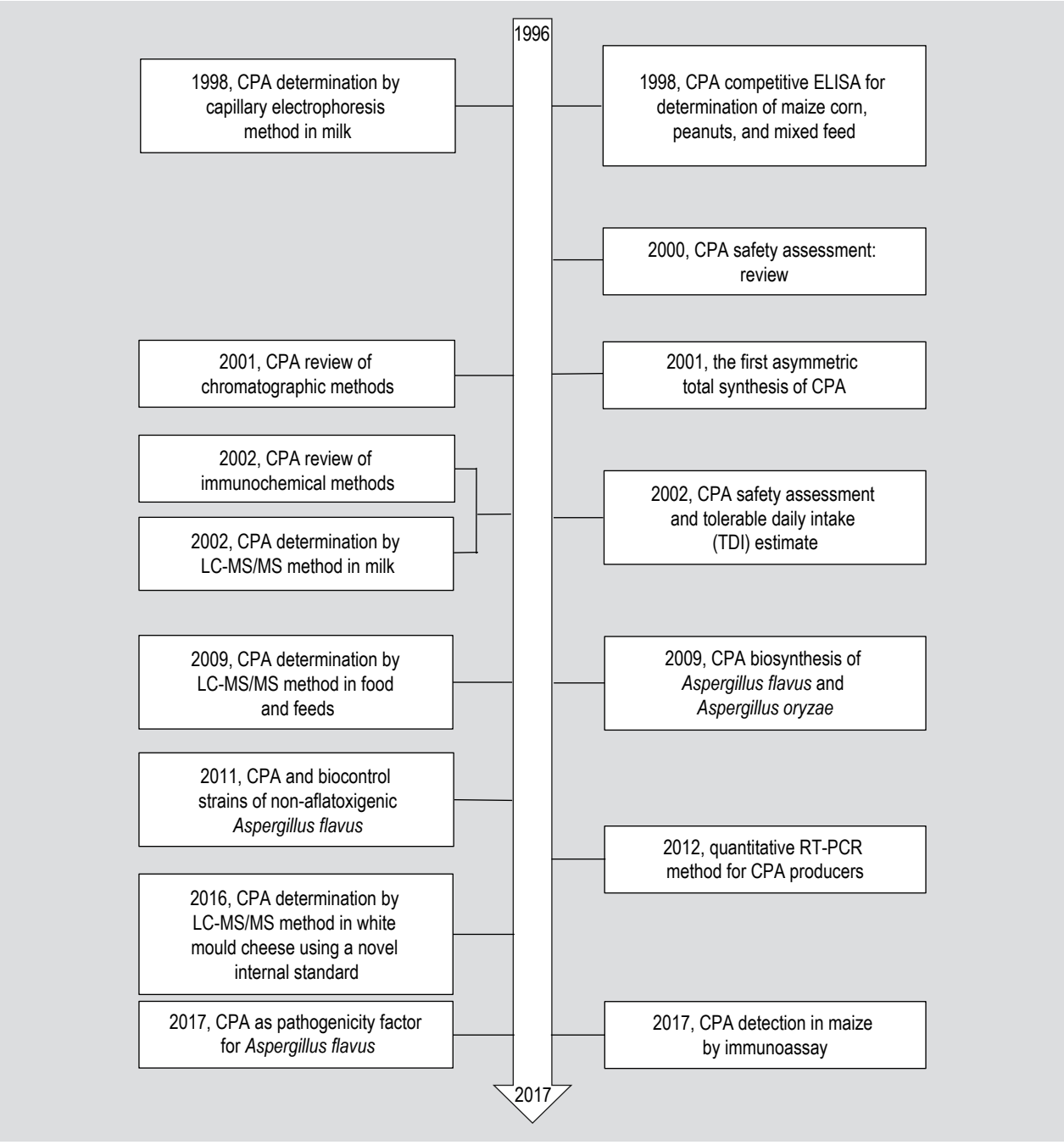
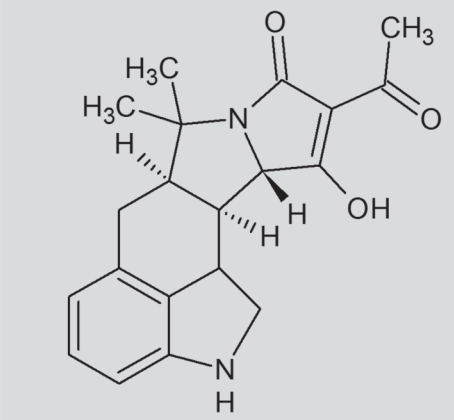


Figure 2. The principal milestones in cyclopiazonic acid (CPA) research in 1996-2017.

P. palitans Westling (synonym of *P. commune*) (Leistner and Pitt, 1977; Pitt *et al.*, 1986), *A. tamarii* Kita (Dorner, 1983) and *A. oryzae* (Ahlb.) Cohn (not all isolates though) (Frisvad, 1989; Orth, 1977) have been identified as CPA-producing strains

Trucksess *et al.* (1987) reported on CPA production by cultures of *Aspergillus* and *Penicillium* species isolated from dried beans, corn meal, macaroni and pecans. El-Banna *et al.* (1987) specifically reported on CPA production by *Penicillium chrysogenum*, *Penicillium nalgiovense*, *Penicillium crustosum*, *Penicillium hirsutum* and *Penicillium viridicatum*, but production by these taxa has not been confirmed.

CPA was shown to be consistently produced in food by *P. griseofulvum*, *P. camemberti, P. commune*, *A. flavus*, *A. oryzae* and *A. tamarii* (Dorner, 1983; Frisvad, 1989; Frisvad, and Samson, 2004). *Penicillium dipodomyicola*, another producer of CPA, has only been found rarely in foods (Frisvad, and Samson, 2004). Other producers of CPA in *Aspergillus* section *Flavi* include *Aspergillus*



PubChem CID: 54682463

CAS (Chemical Abstracts Services) registry no.: 18172-33-3

IUPAC name: (6a*R*,11a*S*,11b*R*)-10-Acetyl-11-hydroxy-7,7-dimethyl-2,6,6a,7,11a,11b-hexahydro-9*H*-pyrrolo[1',2':2,3]isoindolo[4,5,6-*cd*]indol-9-one

Systematic name: 9*H*-Pyrrolo(1',2':2,3)isoindolo(4,5,6-*cd*) indol-9-one,10-acetyl 2,6,6a,7,11a,11b-hexahydro-11-hydroxy-7,7-dimethyl-, (6aα,11aβ,11bα)-(8Cl)(9Cl)

Molar mass: 336.39 g/mol

Exact mass: 336.147 g/mol

Molecular formula: $C_{20}H_{20}N_2O_3$

Figure 3. Chemical structure of cyclopiazonic acid (CPA).

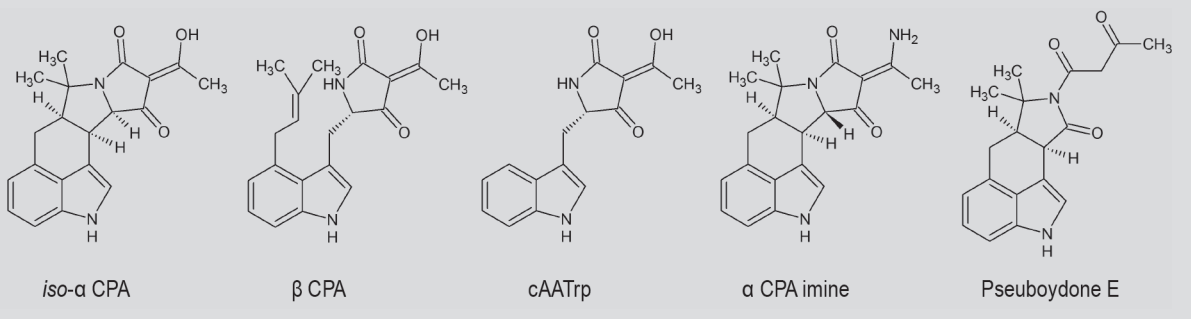


Figure 4. Chemical structures of cyclopiazonic acid (CPA)-type mycotoxins: indole derivatives.

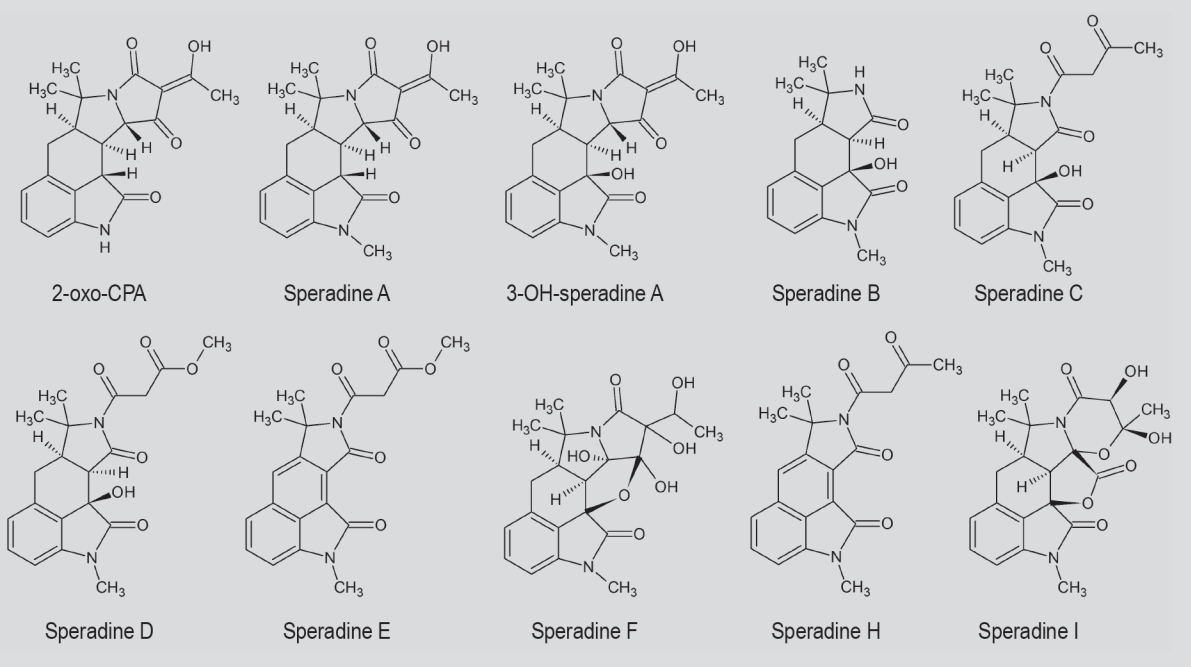


Figure 5. Chemical structures of cyclopiazonic acid-type mycotoxins: speradines.

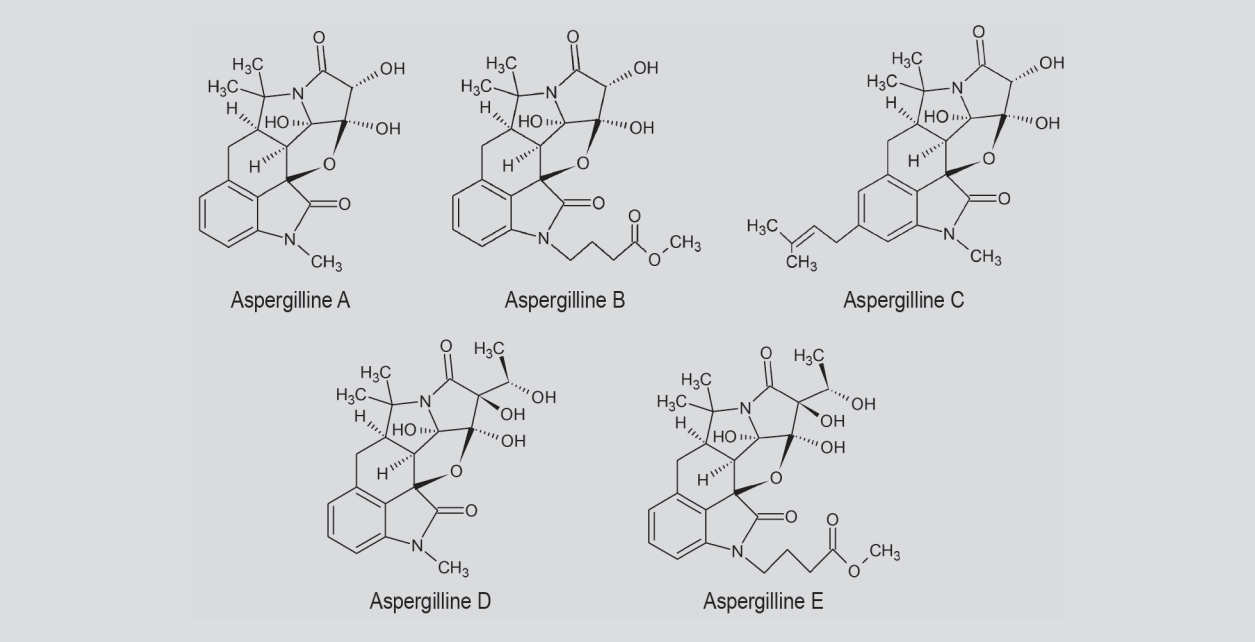


Figure 6. Chemical structures of cyclopiazonic acid-type mycotoxins: aspergillines.

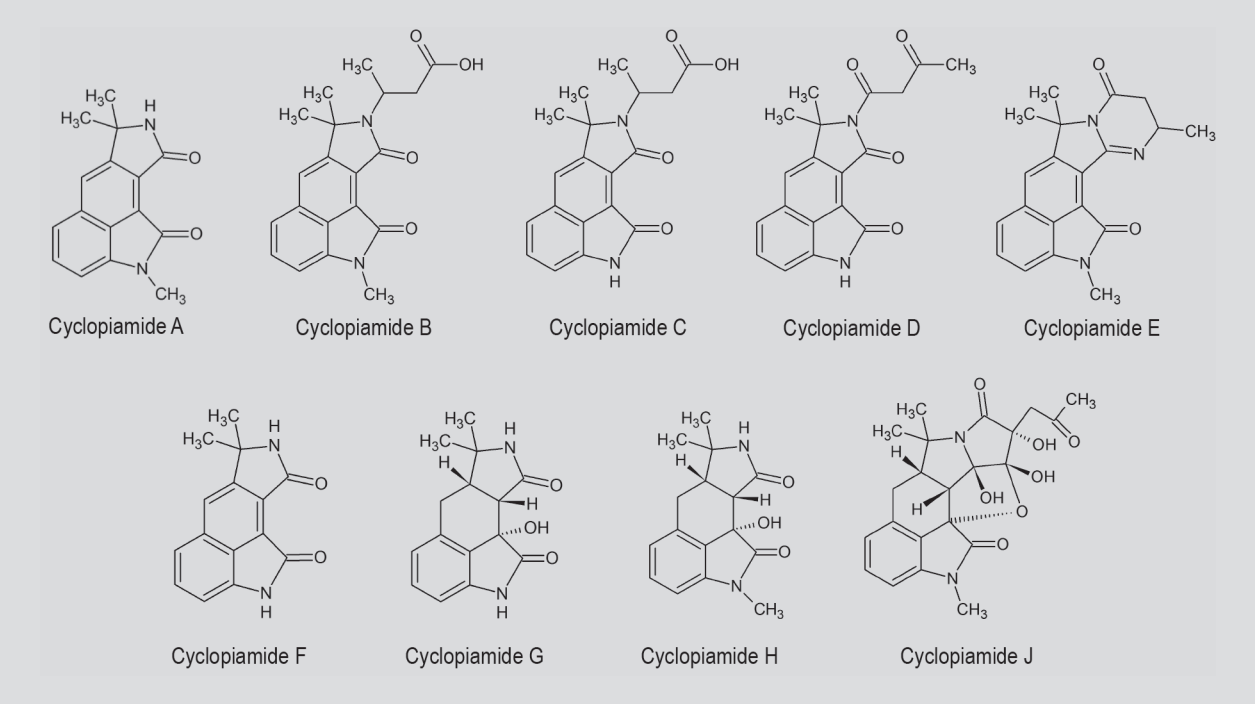


Figure 7. Chemical structures of cyclopiazonic acid-type mycotoxins: cyclopiamides.

parvisclerotigenus (Frisvad *et al.*, 2005), *Aspergillus pseudotamarii* (Ito *et al.*, 2001; Varga *et al.*, 2003; Frisvad *et al.*, 2005) and *Aspergillus minisclerotigenes* (Pildain *et al.*, 2008), but their role concerning CPA production in foods is still unclear (Frisvad *et al.*, 2007; Varga *et al.*, 2011).

In addition to classical mycological methods for detection and toxigenicity testing of CPA producers isolated from foodstuffs, rapid, accurate, specific and highly sensitive quantitative real-time PCR methods to quantify CPA-producing microfungi were necessary. To quantify CPA producing microfungi in foods, Rodríguez *et al.* (2012) reported on a quantitative real-time PCR method with internal amplification control. This method could be used to monitor CPA producers in foodstuffs for effective official food inspection, food monitoring programmes (e.g.

Total Diet Study) and the Hazard Analysis Critical Control Point (HACCP) systems, so that the risk of CPA formation throughout the food chain could be minimised.

4. Cyclopiazonic acid analysis

A variety of analytical methods have been used to detect and quantify CPA in fungal cultures and in food, including chromatographic methods (TLC, HPLC and LC-MS/MS), immunochemical methods (ELISA, immunoaffinity columns (IAC)) and capillary electrophoresis.

Chromatographic methods for CPA were reviewed by Dorner (2002). Most chromatographic methods involve CPA extraction and isolation steps before the determinative step. In most cases, solvents, such as chloroform and dichloromethane were used as extraction and clean-up solvents (Ostry and Polster, 1989; Ostry *et al.*, 1990). To extract CPA from commodities, combinations of acidified chloroform, chloroform/methanol, or mixtures of non-chlorinated solvents (such as aqueous methanol or aqueous acetonitrile) have been used. An alkaline aqueous component is typically used in order to extract the toxin under ionized form, as opposed to acidified chloroform, which is used for extraction of the neutral form. Whether the extraction is alkaline or acidic depends in part upon the downstream clean-up. For example, acidified chloroform has been used together with silica solid-phase extraction or silica minicolumns (Goto *et al.*, 1996; Norred *et al.*, 1987). Recently, acetonitrile:water or methanol:water (7:3, v/v) – with the addition of bicarbonate – has found more widespread use, likely because they avoid the use of halogenated solvents (Aresta *et al.*, 2003; Dorner *et al.*, 2001; Hayashi and Yoshizawa, 2005a,b; Losito *et al.*, 2002; Urano *et al.*, 1992a,b). Samples that have been extracted and isolated are often submitted to a chromatographic step, such as thin-layer chromatography (TLC) (Lansden, 1986; Le Bars, 1979a,b; Ohmomo *et al.*, 1973; Ostry and Polster, 1989; Popken and Dose, 1983; Steyn, 1969) or liquid chromatography (LC) (Aresta *et al.*, 2003; Dorner *et al.*, 2001; Goto *et al.*, 1996; Hayashi and Yoshizawa, 2005a,b; Lansden, 1984; Losito *et al.*, 2002; Norred *et al.*, 1987; Ostry *et al.*, 1990; Urano *et al.*, 1992a,b). The limit of quantification (LOQ) for TLC in foodstuffs was usually around 20 ng/g (Lansden and Davidson, 1983; Le Bars, 1979a; Ostry and Polster, 1989). The LOQ for LC in foodstuffs was usually from one to tens ng/g (Lansden, 1984; Norred *et al.*, 1987; Ostry *et al.*, 1990). Liquid chromatography coupled to various forms of mass spectrometry (LC-MS, LC-MS/MS) is commonly used for mycotoxin detection, and CPA is no exception. The LOQ for LC-MS/MS in foodstuffs was usually from 0.5 ng/g to 1 ng/g (Ansari and Häubl, 2016; Moldes-Anaya *et al.*, 2009; Peng *et al.*, 2017). However, application of the technique to CPA is not without problems (Diaz *et al.*, 2010). Aside from LC-MS/MS methods for CPA, other methods have typically relied upon detection of the absorbance of the toxin in the ultraviolet (UV) range at ~279 nm. Several years ago, it was noted that CPA, which is non-fluorescent, could be rendered fluorescent upon photolysis (Maragos, 2009). This phenomenon led to the development of a HPLC-fluorescence detection (HPLC-FLD) method for the simultaneous detection of aflatoxins and CPA in fungal cultures (Soares *et al.*, 2010).

Besides the above mentioned chromatographic methods, far fewer immunochemical methods have been described. These methods were reviewed by Dorner *et al.* (2001) and include enzyme-linked immunosorbent assay (ELISA) and immunoaffinity column (IAC) formats. Several laboratories had originally developed antibodies directed against CPA (Hahnau and Weiler, 1991, 1993; Yu and Chu, 1998). Maragos *et al.* (2017) recently developed novel monoclonal antibodies that can detect CPA in maize and can be used as components of biosensors for multi-toxin detection; the LOQ for the ELISA method in maize was 5 ng/g. Antibodies of this nature have been applied in both ELISA and IAC formats (Dorner *et al.*, 2001; Hahnau and Weiler, 1991, 1993; Huang and Chu, 1993; Kononenko *et al.*, 2012; Yu and Chu, 1998). Unfortunately, most of the analysts must use complicated isolation and detection procedures, because CPA antibodies and IAC are not available commercially.

A capillary electrophoresis method for quantifying CPA in milk was developed by Prasongsidh *et al.* (1998). The LOQ for this capillary electrophoresis method in milk was not reported in the study, but CPA was detected in spiked milk samples of 20 ng/ml. Fast identification of CPA in goat milk by capillary electrophoresis was reported by Roncada *et al.* (2003). This method is a modification of the method of Prasongsidh *et al.* (1998) and is much easier because raw goat milk artificially contaminated with CPA is only briefly defatted with a micro-centrifuge (12,000 rpm, 10 min, 4 °C) prior to injection into the capillary electrophoresis system. The LOQ was also not specified in this study (Roncada *et al.*, 2003).

5. Occurrence of cyclopiazonic acid in food

There are only a few reports about attempts to quantify CPA contamination in food. CPA occurs in several products of plant origin, such as peanuts (Fernandez Pinto *et al.*, 2001; Lansden, 1986; Lansden and Davidson, 1983; Ostry *et al.*, 1990; Oyedele *et al.*, 2017; Urano *et al.*, 1992a,b; Widiastuti *et al.*, 1988; Zorzete *et al.*, 2013), maize (Hayashi and Yoshizawa, 2005a; Lansden, 1986; Lee and Hagler, 2001; Reddy and Reddy, 1992; Urano *et al.*, 1992a,b), figs (Basegmez and Heperkan, 2015; Heperkan *et al.*, 2012a,b), rice (Goto *et al.*, 1987; Ostry *et al.*, 1989; Rathinavelu and Shanmugasundaram, 1984), groats (Ostry *et al.*, 1989), tomato paste and puree (Da Motta and Soares, 2001), kodo millet (Rao and Husain, 1985), sunflower seeds (Ross *et al.*, 1991) and wheat (Rathinavelu and Shanmugasundaram, 1984).

As for food of animal origin, CPA occurs in cheese (Ansari and Häubl, 2016; Le Bars, 1979a,b; Ostry, 1989; Ostry *et al.*, 1989; Still *et al.*, 1978; Zambonin *et al.*, 2001), milk (Dorner *et al.*, 1994; Oliveira *et al.*, 2006; Prasongsidh *et al.*, 1998) and salami (Ostry *et al.*, 1989).

CPA can be found alone as well as co-occurring with other mycotoxins. For example, CPA was detected together with aflatoxins in maize and peanuts (Urano *et al.*, 1992a,b), with aflatoxins in peanuts (Fernandez Pinto *et al.*, 2001; Soares *et al.*, 2010; Zorzete *et al.*, 2013), with aflatoxins in dried figs (Heperkan *et al.*, 2012a), with tenuazonic acid in cornflakes (Aresta *et al.*, 2003), with aflatoxins, ochratoxin A and zearalenone in Indonesian maize (Widiastuti *et al.*, 1988). CPA was also detected together with aflatoxins, fumonisin B_1 and ochratoxin A in dried figs (Heperkan *et al.*, 2012b). Multi-mycotoxin contamination of groundnut in Nigeria included CPA, aflatoxins (AFB_1, AFB_2, AFG_1, AFG_2 and AFM_1), beauvericin, moniliformin, nivalenol and ochratoxin A; CPA was found in the samples at higher concentrations than the other mycotoxins (Oyedele *et al.*, 2017).

6. Cyclopiazonic acid toxicity

Hazard assessment of cyclopiazonic acid

Several extensive reviews of CPA and CPA toxicology are available (Burdock and Flamm, 2000; Chang *et al.*, 2009b; King *et al.*, 2011; Voss, 1990). CPA is not very acutely toxic (Morrissey *et al.*, 1985; Purchase, 1971; Van Rensburg, 1984). CPA toxicity has been studied in several species including rats (Morrissey *et al.*, 1985; Norred *et al.*, 1985; Purchase, 1971), mice (Nishie *et al.*, 1987), pigs (Lomax *et al.*, 1984), dogs (Nuehring *et al.*, 1985), guinea pigs (Peden, 1990; Richard *et al.*, 1990), monkeys (Jaskiewicz, *et al.*, 1988), chickens (Balachandran and Parthasarathy, 1996; Dorner *et al.*, 1983; Gentles *et al.*, 1999; Kubena *et al.*, 1994; Kumar and Balachandran, 2009; Malekinejad *et al.*, 2011; Norred *et al.*, 1988; Venkatesh *et al.*, 2005a,b), laying hen (Cole *et al.*, 1988) and lactating ewe (Cole *et al.*, 1988).

CPA was added to the category of potentially serious mycotoxins that cause degenerative changes and necrosis in the liver, spleen, pancreas, kidney, salivary glands, myocardium and skeletal muscles, based on toxic effects observed in male and female rats (Cole, 1984; Purchase, 1971). The alimentary tract, liver, kidney, skeletal muscle and the nervous system are the major target organs of toxicity, although the specific response to CPA exposure differs from laboratory animals, such as the rat, mouse and guinea pig, to domestic animals, such as the chicken, pig and dog. CPA causes weight loss, diarrhoea, degeneration and necrosis of the muscles and viscera, and convulsion and death in rodents (rats and mice), dogs and pigs (Voss, 1990). Dorner *et al.* (1983) published the results of a long-term feeding study in broiler chickens. Concentrations of 10, 50, 100 µg/g CPA in the feed were given *ad libitum* to chickens for 7 weeks. Broiler chickens exposed to 50 µg/g developed proventricular lesions characterised by thick mucosa and dilated proventricular lumens. Also frequently observed at necropsy were engorged kidneys in both 50 and 100 µg/g treated groups (Dorner, 1982). A study on the serum levels of divalent cations, on nitric oxide content and mRNA level of inducible nitric oxide synthase in the liver and kidney of CPA-treated chickens, and on the cellular and molecular pathways of CPA toxicity, suggested that CPA-producing fungi along with CPA contamination in the chicken feed result in renal and hepatic disorders (Akbari *et al.*, 2012).

The teratogenic potential of CPA proved to be low in Fischer-344 rats. Nevertheless, significant retardation in embryonic skeletal development (including retardation of ossification of cervical and caudal vertebrae) was evident after oral administration of 5-10 mg/kg body weight (bw) CPA in the feed during pregnancy (Morrissey *et al.*, 1984). Several authors suggested that CPA could be directly toxic to lymphocytes and lymphoid organs, such as the thymus and spleen (Kumar and Balachandran, 2009; Nuehring *et al.*, 1985; Venkatesh *et al.*, 2005a,b), and that CPA, even at very low doses, could induce inflammation in the liver and kidney due to oxidative stress (Malekinejad *et al.*, 2011).

In a French study, CPA cytotoxicity and immunotoxicity were evaluated in human cells *in vitro*. CPA was cytotoxic in human monocytes, CD34+, THP-1 and Caco-2 cells. It was shown, that the THP-1 monocytic cell line was less sensitive to CPA than monocytes after 48 h of incubation in the tested conditions. Under exposure to non-cytotoxic concentrations, human monocyte differentiation into macrophages was impaired (Hymery *et al.,* 2014).

A suggested key mechanism of toxicity is the ability of CPA to modify the normal intracellular calcium flux by the specific inhibition of the sarco(endo)plasmic reticulum Ca^{2+}ATPase (SERCA). SERCA translocates calcium from the cytosol to the endoplasmic reticulum. As such, it has an impact on cell fate and on the necessary environment for enzyme activities. In particular, it plays a substantial role in the muscle contraction-relaxation cycle (Goeger *et al.,* 1988; Riley *et al.,* 1992). CPA has been shown to block calcium access channel and immobilise a subset of four transmembrane helices of the ATPase which may result in the inhibition of the calcium pump (Moncoq *et al.*, 2007). In experimental animals, CPA has also been found to induce various lesions of the lymphoid organs, in particular, of the bursa of Fabricius and spleen. In spite of these findings, CPA does not appear to affect the immune system *in vivo* (Burdock and Flamm, 2000).

There are several reports on the mutagenicity of CPA (Sorenson *et al.*, 1984; Takahashi *et al.*, 1992; Wehner *et al.*, 1978; Yates *et al.*, 1987). Sorenson *et al.* (1984) showed

that CPA was mutagenic to *Salmonella typhimurium* TA98 and TA100 in the presence of metabolic activation. On the other hand, Wehner *et al.* (1978) concluded that CPA was not mutagenic in *Salmonella typhimurium* TA98, TA100, TA1535 and TA1537 with and without metabolic activation by S9 hepatic microsomal fraction. These negative results were later confirmed in studies performed by Yates *et al.* (1987) and Takahashi *et al.* (1992).

The International Agency for Research on Cancer in Lyon (France) – through its IARC Monographs programme – has performed carcinogenic hazard assessment of some mycotoxins in humans on the basis of epidemiological data, studies of cancer in experimental animals and mechanistic studies. CPA has not yet been evaluated by the IARC Monographs programme (Ostry *et al.*, 2017). Based on current mechanistic studies and the lack of adequate data on the carcinogenicity of CPA, it would be difficult to conclude on its genotoxicity and carcinogenicity. As a matter of fact, there are currently no chronic toxicity studies (and few toxicity studies overall) in experimental or domestic animals.

The presence of CPA and aflatoxins in maize and peanuts contaminated with *A. flavus* suggests that synergism may occur. Data from the toxicological evaluations of aflatoxins and CPA in broiler chickens demonstrate that both aflatoxins and CPA alone and the aflatoxins-CPA combination can adversely affect broiler health. In most cases the effects of aflatoxins and CPA were additive (Smith *et al.*, 1992).

Risk assessment of cyclopiazonic acid

'No-observed-adverse-effect-level' (NOAEL), 'lowest-observed-adverse-effect-level' (LOAEL) and benchmark dose (BMD) could not be identified for CPA for any endpoint in published studies. Very few toxicity data on CPA relevant for risk assessment are actually available. Consequently, no risk assessment was performed by the European Food Safety Agency (EFSA) or the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Nevertheless, Burdock and Flamm (2000) proposed an acceptable daily intake (ADI) of 10 µg/kg bw CPA based on a 'no-observed-effect-level' (NOEL) of 1.0 mg/kg bw/day from a study in pigs (Lomax *et al.*, 1984). We note that ADI should only be applied to food additives or residues of a veterinary drug or pesticide in food.

De Waal (2002) responded to the approach of Burdock and Flamm (2000) and proposed a tolerable daily intake (TDI) of 0.1 µg/kg bw/day CPA, based on a NOEL (no observed effect level) of 0.1 mg/kg bw/day derived from the 90-day study in dogs by Nuehring *et al.* (1985). A composite uncertainty factor of 1000 based on (1) uncertainties in the extrapolation from experimental animals to humans, and (2) intra-species variability was applied. For a 70 kg adult, the TDI based on De Waal (2002) proposal would be reached by consuming 50 g per day of maize containing CPA at a concentration of 0.14 µg/g.

There was a revealing exchange in the 'Letter to the Editor – Safety Assessment of CPA' between De Waal and the authors of Burdock and Flamm (2000) in the 'International Journal of Toxicology' (De Waal, 2002) in which each group defended their interpretation of the critical study they used to establish their recommendation. From their exchange, we conclude there was no basis upon which to set a TDI due to the absence of adequate data. Overall, we estimate that data from relevant sub-chronic studies on CPA in experimental animals are inadequate to determine a TDI.

7. Recent research topics on cyclopiazonic acid

The aim of this review was not to incorporate all research topics associated with CPA. Nevertheless, it is worth noting selected recent research topics such as:

- Research on the diversity and genetic variability of CPA production in *A. flavus* and on the problem of biocontrol strategy for use of the non-aflatoxigenic strains of *A. flavus* (Abbas *et al.,* 2011; Astoreca *et al.*, 2014; Barros *et al.*, 2006; Chang *et al.,* 2009a, 2012; Horn and Dorner, 1999; Payne *et al.,* 2011; Solorzano *et al.*, 2014).
- CPA as a pathogenicity factor for *A. flavus* (Chalivendra *et al.,* 2017).
- The enhancing effect of the food additive potassium sorbate at 0.02% on the production of CPA by *P. commune* (Zhelifonova *et al.*, 2017).

8. Conclusions

CPA is a potentially serious mycotoxin and its toxicity to several animal species warrant further chronic toxicity and carcinogenicity studies. The results of chronic toxicity and carcinogenicity studies, recent consumption data, and the occurrence of CPA in foodstuffs are required for the assessment of toxicity severity and estimation of human dietary exposure and health risk assessment.

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