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Ochratoxin A – Toxicological Aspects

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Abstract. European and national public authorities are introducing increasingly high standards to ensure that health risks, induced by contaminated food, is prevented and considerable reduced. This is olso the objective for the agribusiness and the distributors; they are the responsibility to guarantee that food is safe. Preventing the risks of contamination by bacteria, pesticides, chemicals, mycotoxins, etc. in food products is a major challenge for all stakeholders in agrifood supply chains. Cases of food poisoning have always hit the headlines and can have very serious economic consequences. Aflatoxins, fumonisins, trichotecenes, zearalenones and ochratoxins belonging to the mycotoxins group are responsible for extremely serious human pathologies. Ochratoxin A, or OTA, produced by fungi of the genera Aspergillus and Penicillium, is acknowledged to be responsible for certain kidney pathologies in pigs and is suspected of causing some human kidney pathologies. It has been shown in rats that OTA has carcinogenic and immunotoxic properties. Because OTA has a long biological cycle, remaining in the bloodstream and contaminating the meat from animals having ingested it, just as it can contaminate mother's milk.

Keywords: mycotoxins, Ochratoxin A, risks of contamination, food safety.

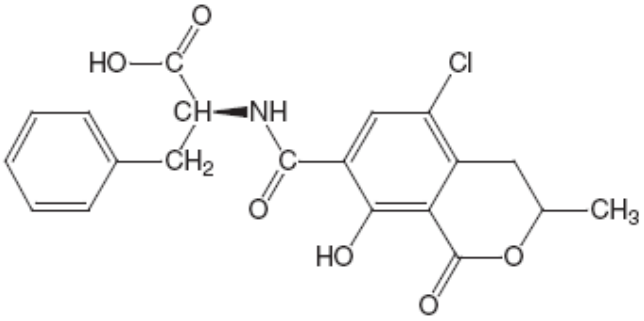
INTRODUCTION

Ochratoxins are a group of structurally related secondary metabolites, that are produced by some toxic fungi such as *Penicillium Verrucosum* and b*y Aspergillus ochraceus;* occasionally also isolates of the common species *Aspergillus niger* can produce Ochratoxin A (OTA) [1]. OTA is the main mycotoxin in the group of Ochratoxins, and it appears to be the only one of toxicological significance. OTA is generally found in cereals, oleaginous seeds, green coffee, pulses, wine, and poultry meat. OTA production depends on both environmental and processing conditions (climatic conditions, abnormally long storage, transportation, wet or dry milling, roasting procedures, fermentation etc.) [1, 8].

Ochratoxin A is recognized to bee a human carcinogen based on sufficient evidence of carcinogenicity in experimental animals [2-4]. When ochratoxin A was administered in the diet, hepatocellular tumors, renalcell tumors, hepatomas and hyperplastic hepatic nodules were observed in male mice. It was shown that administration of ochratoxin A in the diet induced hepatocellular carcinomas and adenomas in female mice. No adequate human studies of the relationship between exposure to ochratoxin A and human cancer have been reported. Incidence of and mortality from urothelial urinary tract tumors have been correlated with the geographical distribution of Balkan endemic nephropathy in Bulgaria and Yugoslavia. [7, 9] A relatively high frequency of contamination of cereals and bread with ochratoxin A has been reported in an area of Yugoslavia where Balkan endemic nephropathy is present [IARC 1987, 1993].

Sources and properties

Ochratoxin A is a toxic metabolite produced primarily by *Aspergillus*, but also by *Penicillium* and other molds. It is a white crystalline powder. Recrystallized from xylene, it forms crystals that emit green (acid solution) and blue (alkaline solution) fluorescence in ultraviolet light; the melting point of these crystals is 169ºC. The free acid of ochratoxin A is soluble in organic solvents [5]. The sodium salt is soluble in water. Ochratoxin A is unstable to light and air, degrading and fading even after brief exposure to light, especially under humid conditions. Ethanol solutions are stable for longer than 1 year if kept refrigerated and in the dark. Ochratoxin A is fairly stable to heat; in cereal products, up to 35% of the toxin survives autoclaving for up to 3 hours [5].



Biosynthesis of OA

Ochratoxin A is naturally produced by fungi. The most important ochratoxin A producing species is *A. ochraceus*. It is also produced by one species of *Penicillium, P. verrucosum*, and rare species in the *ochraceus* group [IARC 1993].

The effects of water activity (a_w) and temperature, the main factors controlling mycotoxin formation, have been elucidated for three fungal organisms: *A. ochraceus*, P. cyclopyium, and P. viridicatum. The minimum a_w values for Ochratoxin production are 0.83-0.87, 0.87-0.90, and 0.83- 0.86 respectively. At 24°C, optimum a_w values for *A. ochraceus* are 0.99 and for both *Penicillium* fungi are 0.95-0.99. At optimum a_w, the temperature range for OA production by *A ochraceus* is 12- 37°C, whereas that for *Penicillium* ones is 4-31 °C [5]. The biosynthesis of OA has been studied using both 14C- and 13C- labelled precursors. DL-[1- 14C]-α-phenylalanine was incorporated into OA by cultures of *A. ochraceus*. Hydrolysis of the labelled OA gave the isocoumarin acid and L-phenylalanine with the amino-acid containing all the activity. The isocoumarin acid derived upon acid hydrolysis of OA contained all activity. Kuhn- Roth oxidation and subsequent Schmidt degradation of the acetic acid provided evidence for the pentaketide origin of the dihydroisocoumarin moiety. The origin of the carboxy group at C (8) was established through the addition of DL-[methyl-14C] methionine to a resting culture of *A. ochraceus* and selective degradation experiments established that the C (12) was derived from methylmethionine [3].

Toxicity

Ochratoxin A (OTA) has been shown to be nephrotoxic, hepatotoxic, teratogenic and immunotoxic to several species of animals and to cause kidney and liver tumours in mice and

rats [4]. As far as humans are concerned, the IARC (International Agency for Research on Cancer) classified OTA as a possible carcinogen to humans (Group 2B) [4]. With regard to nephrotoxicity, OA is considered to be involved in the Balkan Endemic Nephropaty (BEN), severe kidney pathology, generally occurring in some areas of South-Eastern Europe (Bosnia, Croatia, Bulgaria and Romania) andlinked to urinary tracts tumours [7]. As regards to the toxicity of OTA, it was observed that its biotransformation is cytochrome P450 dependent in animals and humans, and it results in the formation of metabolic intermediates active in the carcinogenic and other toxic activities. The cytochrome stimulates the OTA induced lipids peroxidation, this process involves moreover some enzymes, present in the cell, and leads to the formation of toxic metabolites [13]. Actually the metabolites of OTA involved in its genotoxic power are not exactly known, but it is demonstrated that dietary feeding of this toxin induces renal adenomas and hepatocellular carcinomas in mice and in rats, and it is suspected for humans.

As regards oral LD_{50} values, they are 20 mg/kg and 3.6 mg/kg in young rats and in day-old chicks, respectively; OTA is also lethal to mice, trout, dogs and pigs [13]. The complex toxic activity of OTA is multifaceted in relation to the role of one of its structural components, L-phenylalanine, which is involved in the inhibition of numerous reactions where it is known to function. The covalent bonds of chemical substances, or their metabolites, to DNA are considered a key step in the processes which induces to carcinogenesis. OTA, as not ionized form, is passively absorbed in the gastrointestinal tract and through enterohepatic circulation can undergo secretion and reabsorption [10]. Moreover the absorption of this mycotoxin occurs in the kidney proximal and distal tubules. It has been observed that OTA induces the formation of several DNA-adducts, because of its chemical structure, in many tissues. These ones are generally repaired in a few times, but in kidney they are still present after 16 days [9]. OTA mean-life is longer than 500 hours, as it was established according to some studies concerning the metabolism of the toxin in monkeys, which are the animal species the most similar to humans [13].

Recommendations for maximum exposure

The Joint FAO/WHO Expert Committee on Food Additives (JEFCA), on the basis of the nephrotoxicity of OA, proposed a provisional tolerable weekly intake (PTWI) for OA of 0.1 μg/kg body mass (equivalent to 14 ng/kg body mass/day) [16]. However on the basis of carcinogenity data, The Working Group of the Nordic Council of Ministers proposed a maximum tolerable daily intake of 5 ng/kg bw of toxin, similar to the provisional tolerable daily intake (PTDI) established by the Canadian authority (1.2 - 5.7 ng/kg bw) [16]. In 1998, taking into account the SCOOP data, the Scientific Committee for Food of the European Commission suggested that it was prudent to reduce exposure to OTA as much as possible, “ensuring that exposures are towards the lower end of the range of tolerable daily intakes of 1.2-14 ng/kg bw/day which have been estimated by other bodies, e.g. below 5 ng/kg bw/day” [14, 15].

Ochratoxin A is a naturally occurring mycotoxin. It exists completely in particulate phase in ambient atmosphere. It is immobile in soil. Its widespread occurrence in food and animal feed results in probable human exposure. Mycotoxins may well be among the world’s most significant food contaminants [6].

Ochratoxin-producing fungi are included in the *Penicillium* and *Aspergillus* genera [8]. In the colder climates, ochratoxin A is formed by *Penicillium* strains; while in tropical and subtropical areas, ochratoxin A is formed by *Aspergillus*. Ochratoxin A is a natural

contaminant on corn, peanuts, storage grains, cottonseed, and decaying vegetation [12]. It has been detected in moldy cereals including wheat, maize, rye, barley, and oats; peanuts; coffee beans; bread; flour; rice; peas; and beans [8]. Detected contamination levels in cereals range from 0.03 to 27.5 ppm [8]. Although the carryover from barley into beer is possible, one survey of all 130 U.S. breweries failed to detect ochratoxin A (up to 10 μg/kg) in beer or malted barley [11]. Residues of ochratoxin A have been detected in samples of meat from animals slaughtered immediately after consuming contaminated feed [14]. It has been detected at levels of 10 to 920 μg/kg in sausage, ham, and bacon samples [14]. No direct evidence of worker exposure has been reported. Potential worker exposure exists for all personnel handling and storing grains, nuts, corn, cereals, and animal feeds.

CONCLUSIONS

- No adequate human studies of the relationship between exposure to ochratoxin A(OTA) and human cancer have been reported;
- Incidence of, and mortality from urothelial urinary tract tumors have been correlated with the geographical distribution of Balkan endemic nephropathy in Bulgaria and Yugoslavia;
- A relatively high frequency of contamination of cereals and bread with ochratoxin A has been reported in an area of Yugoslavia where Balkan endemic nephropathy is present;
- Many countries set specific regulations for OTA at levels ranging from 1 to 50 μg/Kg for foods, and from 5 to 300 μ g/Kg for animal feeds;

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