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Ochratoxin A: Previous risk assessments and issues arising

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Abstract
Ochratoxin A (OTA) causes nephropathy in all species tested with large sex and species differences in potency, pigs being most sensitive. It has been linked to Balkan endemic nephropathy (BEN) in humans. Embryotoxicity, teratogenicity, and immunotoxicity occur only at doses higher than those causing nephrotoxicity. OTA has long serum half-lives in various species including humans. OTA produced renal tumours in mice and rats. The male rat was most sensitive, renal carcinomas occurring after 70 μg/kg bw per day but not 21 μg/kg bw per day. OTA was not mutagenic in most studies in bacteria and mammalian cells, but produced DNA damage and chromosomal aberrations in mammalian cells *in vitro*, and in mice *in vivo*. DNA adducts found in the kidneys of mice and rats dosed with OTA, did not contain fragments of OTA. OTA in food has been evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), and by the EC Scientific Committee on Food (SCF). JECFA established a provisional tolerable weekly intake (PTWI) of 100 ng/kg bw based on the LOEL for renal effects in pigs. Conversely, SCF recommended reducing exposure to OTA as much as possible, e.g. below 5 ng/kg bw per day. Both committees recommended further studies to clarify the mechanism by which OTA induces nephrotoxicity and carcinogenicity.

Keywords: *Ochratoxin A, toxicity, risk assessment*

Introduction

Ochratoxin A (OTA) is a mycotoxin produced by *Penicillium verrucosum* and by several species of the genera *Aspergillus*. OTA occurs in cereals and cereal products, coffee, beans, pulses, grapes, wine, and dried fruits. As cereals are widely used in animal feed, and because OTA is relatively stable *in vivo* it can also be found in some animal products, especially in pig kidney and liver. OTA has been causally associated with nephropathy in pigs and poultry. In humans, intake of high amounts of OTA has been linked to Balkan endemic nephropathy (BEN), a chronic nephropathy described in several rural regions of Bulgaria, Romania, Serbia, Croatia, and Bosnia, and associated with an increased incidence of tumours of the upper urinary tract. However, causality has not yet been established.

Several international expert groups, such as the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (WHO 1991, 1996, 2001) and the former European Commission Scientific Committee on Foods (SCF 1996, 1998), have evaluated Ochratoxin A in food. In all cases effects on the kidneys (nephropathy in all species investigated and renal carcinogenicity in rodents) were considered as pivotal.

The purpose of this paper is to briefly review these previous risk assessments of OTA in order to highlight the critical issues that these assessments had identified. Unless otherwise stated the primary source has been the last JECFA evaluation (WHO 2001). No new information since that time has been included in this paper as this will be dealt with in the subsequent papers from the Workshop.

Absorption, distribution, metabolism and excretion

OTA is readily absorbed from the upper gastrointestinal tract, in particular from the small intestine. The overall percentages found to be absorbed were 66% in pigs, 56% in rats, 56% in rabbits, and 40% in

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chickens. In blood, OTA is bound to serum albumin and other macromolecules. Only 0.02% remained unbound in rats and humans. In pigs, rats, chickens, and goats OTA was mainly distributed to the kidneys with lower concentrations being found in liver, muscle, and fat. In rats and pigs, OTA crossed the placenta, and was found to be transferred to the milk in rats, rabbits, and humans.

Disappearance of OTA from blood was slower than from kidney, liver, and other tissues in pigs. Serum half-lives after oral administration of OTA were 510 h in one monkey, 72–120 h in pigs, 55–120 h in rats, 24–39 h in mice, and 4.1 h in chickens. In one human volunteer, the half-life was 840 h (about 35 days).

OTA is excreted into bile and urine. In all species examined, OTA is hydrolyzed (detoxified) to ochratoxin α, mainly by the bacterial microflora in the intestine. The capacity of the liver and kidney to hydrolyse OTA is low. However, OTA that is excreted into the bile can be hydrolyzed by the intestinal microflora and the resultant ochratoxin α may be absorbed. In this way OTA undergoes entero-hepatic circulation. OTA has been shown to be oxidized to 4-OH-ochratoxin A (4R and 4S) epimers by cytochrome P450s in liver microsomes from mice, rats, pigs, and humans *in vitro*. All the metabolites are considered to be less toxic than OTA itself. In rats given radiolabelled OTA, 33% of the radiolabel was found in faeces, and the major excretory products in urine of rats were ochratoxin α, OTA, and 4R-OH-ochratoxin A (26%, 6%, and 1.5%, respectively).

Acute, short- and long-term toxicity

Acute lethal doses of OTA produce widespread multifocal haemorrhages, intravascular coagulation and necrosis of liver, kidney and lymphoid organs. There is a wide variation in species sensitivity, with dog and pigs being more sensitive than mice and rats. Oral LD_{50} values were: dog 0.2 mg/kg bw; pig 1 mg/kg bw: rat 20–30 mg/kg bw and mouse 46–58 mg/kg bw.

OTA is a potent renal toxicant in all animal species tested. In short-term studies, rats, dogs, and pigs have shown dose- and time-dependent development of progressive nephropathy histologically characterized by karyomegaly and necrosis of tubular cells, and thickening of tubular basement membranes. The target site is specific being the straight segment of the proximal tubule S3 in the outer stripe of the outer medulla. Significant sex and species difference have been observed in the sensitivity to the nephrotoxic action of OTA, with pigs being more sensitive than rats and mice. In pigs, a Lowest Observed Effect Level (LOEL) for effects on the kidneys (effects on enzymes and function) was established at 8 μg/kg bw per day in a 90-day feeding study. However, this dose was described as a No Observed Effect Level (NOEL) in chronic (2-year) feeding studies in pigs in which progressive nephropathy was observed after 40 μg/kg bw per day.

OTA was embryotoxic and teratogenic in rats and mice after administration by oral gavage. In mice, craniofacial defects were seen after 3–4 mg/kg bw on days 8–9 of gestation and skeletal and renal defects were seen in rats at 1 mg/kg bw per day on days 6–15 of gestation. Learning deficits have been reported in offspring of rats after gavage doses of 0.5 mg/kg bw per day to the dams on days 11–14 of gestation. Immunosuppressive effects have also been observed in several species (mice, rats, pigs, and chickens), but functional deficits have been observed only after parenteral administration or at oral doses higher than those that cause nephrotoxicity.

OTA also elicited neurotoxicity in rats following intracerebral injection (400 ng per animal) or by gavage (250 μg/kg bw) every 48 h for six weeks. These studies are not suitable for establishing a NOEL.

OTA has been tested for chronic toxicity and carcinogenicity in rats and mice in 2-year dietary studies. Considerable species and sex differences in sensitivity were observed both in relation to nephrotoxicity and tumour induction. A dose-related increased incidence of benign and malignant renal tumours originating at a specific site, the proximal tubule segment S3, was observed in male and female rats and in male mice. The tumours were of an aggressive nature with metastasis in various other organs. The male rat was most sensitive with renal carcinomas found in 16/51 rats at 70 μg/kg bw per day and 30/50 at 210 μg/kg bw per day. No carcinomas were found at the lower dose of 21 μg/kg bw per day (see Table I). Renal adenomas, as well

Table I. Renal tumours and karyomegaly in male rats in the NTP study (1989).

Dose (μg/kg bw/day)	Adenomas	Carcinomas	Adenomas + carcinomas	Karyomegaly
0	1/50	0/50	1/50	0/50
21	1/50	0/50	1/50	1/50
70	6/51	16/50	20/51	51/51
210	10/50	30/50	36/50	50/50

Table II. LOELs and NOELs for nephrotoxicity and carcinogenicity of OTA.

Species	Effect	Study duration	LOEL (μg/kg bw/day)	NOEL (μg/kg bw/day)
Mouse (male)	Kidney tumours	2 years	4,400	130
Rat (male)	Karyomegaly of proximal tubule cells	90 days	15	Not established
	Kidney tumours	2 years	70	21
Pig	Impaired renal function	90 days	8	Not established
	Progressive nephrophathy	2 years	40	8

as nephropathy and karyomegaly were found in all dosed groups. A slight increase in liver tumours has been reported in mice given high doses of OTA.

An overview of the NOELs and LOELs for the nephrotoxicity and renal carcinogenicity of OTA is given in Table II.

Most studies on gene mutations in bacteria and mammalian cells were negative. However, OTA produced DNA damage, DNA repair, and chromosomal aberrations in mammalian cells *in vitro*, and DNA damage and chromosomal aberrations in mice *in vivo*. DNA adducts (spots) have been found using a ^{32}P-postlabelling method in kidneys of mice and rats given OTA, but the adducts have so far not been demonstrated to contain OTA or fragments of OTA, and experiments using radiolabelled OTA did not demonstrate formation of covalent DNA-adducts from OTA.

In the absence of evidence that the genotoxicity of OTA *in vitro* and *in vivo* was mediated by formation of a reactive intermediate of OTA or by direct interaction with DNA it has been speculated that OTA may act as a genotoxin by generating reactive oxygen species or other, indirect epigenetic mechnisms.

In 1993 the International Agency for Research on Cancer (IARC) classified OTA as a possible human carcinogen (group 2B), based on sufficient evidence for carcinogenicity in animal studies and inadequate evidence in humans (IARC 1993).

Mechanism of action

A primary effect of OTA is inhibition of protein synthesis; secondarily, RNA and DNA synthesis may be inhibited. *In vitro*, OTA competitively inhibits phenylalanine-tRNAPhe synthetase, so that aminoacylation and peptide elongation are stopped. The effect can be reversed by increased phenylalanine concentrations *in vitro*, and acute effects *in vivo* can be counteracted by phenylalanine or aspartame.

However, the carcinogenic mode of action of OTA was unclear at the last JECFA (2001) and SCF (1998) reviews, but several mechanism have been postulated:

- Genotoxicity (evidence equivocal)
- Secondary to chronic renal cytotoxicity
- Secondary to inhibition of phenylalanine-tRNAPhe synthetase and protein synthesis
- Mitochondrial dysfunction and oxidative stress.

International risk assessments of OTA

JECFA

At the 37th meeting (WHO 1991) a provisional tolerable weekly intake (PTWI) of 112 ng/kg bw per week was established based on the LOEL of 0.008 mg/kg bw per day for deterioration of renal function in pigs to which a safety factor of 500 was applied. At the 44th meeting (WHO 1996) the PTWI was reconfirmed but rounded to 100 ng/kg bw per week. The PTWI was retained at the 56th meeting (WHO 2001), the last time JECFA evaluated OTA. JECFA established the provisional tolerable intake of OTA on a weekly basis rather than on a daily basis because of its long elimination half-life in humans.

JECFA in its latest evaluation (WHO 2001) concluded that the mechanism by which OTA causes nephrotoxicity and carcinogenicity was unknown, although both genotoxic and non-genotoxic modes of actions had been proposed. JECFA considered that the nephrotoxicity preceded the carcinogenicity in the rat, because tumours were only observed at nephrotoxic doses, and frank nephrotoxicity was occurring long before the onset of the carcinogenicity, as found in short-term studies (16 days and 13 week) that were parallel to the long-term carcinogenicity study by the NTP (1989). In addition, the renal tumour development was clearly related to the site of OTA-induced tubule damage, as preneoplastic hyperplasia, adenomas, and early carcinomas developed within the outer stripe of the outer medulla. Therefore, JECFA confirmed its earlier evaluation. JECFA also noted the large effective safety factor of 1300 on the NOEL of 21 μg/kg bw per day for renal tumours in the male rats.

Although an association between intake of OTA and Balkan endemic nephropathy in humans has been postulated, causality has not been established. Based on food consumption in Europe, JECFA in 2001 estimated the mean total OTA intake to be

45 ng/kg bw per week, assuming a body weight of 60 kg. The estimated intake by the 95th percentile consumer of cereals alone would approach the PTWI (WHO 2001).

SCF

In 1994, the SCF considered OTA to be a potent nephrotoxic agent, a carcinogen and to have genotoxic properties. As a provisional conclusion the SCF stated that an acceptable level of daily exposure would fall in the range of a few ng/kg bw per day (SCF 1996). In 1998, the SCF stated that OTA possesses carcinogenic, nephrotoxic, teratogenic, immunotoxic and possibly neurotoxic properties. It was considered negative in conventional mutagenicity tests. However, *in vitro* and *in vivo* tests using less conventional methods have provided evidence of the genotoxic potential of OTA. The SCF considered it 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 per day which has been estimated by other bodies, e.g. below 5 ng/kg bw per day'' (SCF 1998). Although the SCF stated that ''there is now increasing concern about the potential genotoxicity of ochratoxin A and its mechanism'', the basis for the SCF evaluation is not particularly transparent, since it is not clear if this is a TDI, what calculation was used to derive the figure nor whether this is optimal or arbitrary.

Others

The Canadian Authorities have also evaluated OTA several times, and suggested a Provisional Tolerable Daily Intake of 1.2–5.7 ng OTA/kg bw per day for a lifetime risk level of 10^{-5}. The evaluations were based on the carcinogenic properties of OTA and both a safety factor- and model-based approaches were used (Kuiper-Goodman 1996). A Nordic expert group evaluated OTA in 1991 (NNT 1991). Several model-based approaches were applied on the tumour data for male and female rats using risk levels of 10^{-5} and 10^{-6} and overall the group proposed a Highest Tolerable Daily Intake of 5 ng/kg bw per day for OTA.

Issues arising

Both JECFA and SCF recommended that studies should be conducted to clarify the mechanism by which OTA induces nephrotoxicity and carcinogenicity, and that epidemiological studies should be encouraged to explore the role of OTA in chronic renal disease. In addition, JECFA recommended that appropriate sampling procedures for foods likely to be contaminated by OTA should be developed, and that better surveys were needed, in particular outside Europe, to assess OTA intake.

The evaluation of further studies into the mode of action of OTA performed after the last JECFA evaluation is one of the main topics of this workshop.

References

IARC 1993. Monographs on the evaluation of carcinogenic risk to humans; Vol. 56: Some naturally occurring substances, food items and constituents, heterocyclic aromatic amines and mycotoxins. International Agency for Research on Cancer, World Health Organization. Lyon, France. pp 489–521.

Kuiper-Goodman T. 1996. Risk assessment of ochratoxin A: An update. Food Additives and Contaminants 13 (Suppl.):53–57.

NNT 1991. Health evaluation of ochratoxin A in food products. The Nordic Working Group on Food Toxicology and Risk Evaluation, Nordiske Seminar- og Arbejdsrapporter 1991:545. Nordic Council of Ministers, Copenhagen, Denmark.

SCF 1996. Opinion on aflatoxin, ochratoxin A and patulin, expressed on 23rd September 1994. Reports of the Scientific Committee for Food, 35th series, Luxembourg.

SCF 1998. Opinion on ochratoxin A. Expressed on 17 September 1998, the Scientific Committee on Food, the European Commission. http://europa.eu.int/comm/food/fs/sc/scf/out14_en.html

WHO 1991. Ochratoxin A. In: Toxicological evaluation of certain food additives and contaminants, Prepared by the 35th Meeting of the Joint FAO/WHO Expert Committee on Food Additives. WHO Food Additives Series, 28. Geneva: World Health Organisation. pp 365–417.

WHO 1996. Ochratoxin A. In: Toxicological evaluation of certain food additives and contaminants, Prepared by the 44th Meeting of the Joint FAO/WHO Expert Committee on Food Additives. WHO Food Additives Series, 35. Geneva: World Health Organisation. pp 363–376.

WHO 2001 Ochratoxin A. In: Safety evaluation of certain mycotoxins in food, Prepared by the 56th Meeting of the Joint FAO/WHO Expert Committee on Food Additives. WHO Food Additives Series, 47. Geneva: World Health Organisation. pp 281–387.