

Opinion of the Scientific Committee on Food on Ochratoxin A (expressed on 17 September 1998)

1. Terms of reference

The Committee is asked to review and revise as necessary its opinion of 22 September 1994¹ on ochratoxin A in the light of results of toxicological studies published since that time.

In making its review the Committee is asked to take note of the recently published Scientific Co-operation report "Assessment of the dietary intake of ochratoxin A by the population of the EU Member States"².

2. Background

Ochratoxin A is a mycotoxin produced by several fungi (*Penicillium* and *Aspergillus* species), and occurs naturally in a variety of plant products such as cereals, coffee beans, beans, pulses and dried fruit all over the world. It has been detected also in products such as coffee, wine, beer and grape juice. It occurs also in kidney, liver and blood from mammals by transfer from animal feed. Investigations of the frequency and levels of occurrence of ochratoxin A in food and human blood samples indicate that foodstuffs are frequently contaminated.

Ochratoxin A is a nephrotoxic mycotoxin which is carcinogenic to rodents and possesses teratogenic, immunotoxic^{3 4 5} and possibly neurotoxic^{6 7} properties. Further, it may be implicated as a factor in the human disease Balkan Endemic Nephropathy and the development of urinary tract tumours in humans^{8 9}. Also, recent data from France and North Africa point towards a correlation between chronic interstitial nephritis and high exposure to ochratoxin A^{10 11 12 13 14}.

In its opinion in 1994 the Committee stated that ochratoxin A is a potent nephrotoxic agent, a carcinogen and that it has genotoxic properties. The genotoxic effect may be explained by an indirect mechanism involving impaired protein synthesis. The Committee provisionally concluded that an acceptable safe level of daily exposure would fall in the range of a few ng/kg b.w./day and it proposed to reconsider its opinion in the light of new information¹.

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has evaluated ochratoxin A at its 37th meeting in 1991¹⁵ and at the 44th meeting in 1995¹⁶. In its assessments, JECFA addressed the carcinogenic effect, but based its assessments on the nephrotoxic effect in pigs (the most sensitive species). With a Lowest Observed Adverse Effect Level (LOAEL) of 8 µg/kg b.w. and applying a safety factor of 500, the Committee in 1991 arrived at a Tolerable Daily Intake (TDI) of 16 ng/kg b.w., which was converted to a Provisional Tolerable Weekly Intake (PTWI) of 112 ng/kg b.w.. This value was rounded off to 100 ng/kg b.w. at the 1995 meeting (i.e. not a change in the toxicological evaluation).

The Canadian authorities have evaluated ochratoxin A in 1989, '90, '91 and '96^{17 18 19 20} and suggested a Provisional Tolerable Daily Intake (PTDI) of 1.2-5.7 ng ochratoxin A/kg b.w./day for a lifetime risk level of 10⁻⁵. The evaluations were based on ochratoxin A's carcinogenic properties and both a safety factor- and model-based approach were used in the calculations.

A Nordic expert group on food toxicology made an assessment in 1991 and proposed a highest tolerable daily intake of 5 ng/kg b.w./day, based on the carcinogenic properties of ochratoxin A. Model-based approaches were used in the calculations²¹.

In 1993, the International Agency for Research on Cancer (IARC) classified ochratoxin A as a possible human carcinogen (group 2B), based on sufficient evidence for carcinogenicity in animal studies and inadequate evidence in humans²².

Since many reviews on ochratoxin A are available and there is general agreement about the toxicity profile, the present report focuses primarily on the mode of action of ochratoxin A carcinogenicity.

3. Exposure

An assessment of dietary intake of ochratoxin A by the population of EU Member States was published² in the framework of the Scientific Co-operation²³ of the European Commission. Thirteen countries provided data on occurrence of ochratoxin A in food products, on consumption of these food products and on occurrence of ochratoxin A in human blood plasma and human milk.

There were large differences in the amount, detail and quality of the data from the participating countries; the judgement as to whether the occurrence data were representative or not, and thus relevant for the intake estimations, was made by the participating countries for their own data. Eight countries were able to estimate mean dietary intake for an average adult person based on food occurrence and consumption data, and these mean dietary intakes were in the range from 0.7 to 4.6 ng/kg b.w./day. Of these eight, five also gave an estimate of mean dietary intake for an average adult person based on human blood plasma data, and these were in the range from 0.2 to 2.4 ng/kg b.w./day. These values corresponded to average blood plasma concentrations of 0.18 to 1.8 ng/ml, respectively. Thus, all the estimates of mean dietary intakes of ochratoxin A for average adult persons fell in the range from close to zero to a few ng/kg b.w./day. Since the dietary intake data are mean values it is understood that some individuals will be exposed to higher levels of ochratoxin A.

The main contributor to the dietary intake of ochratoxin A seems to be cereals and cereal products.

4. Evaluation of the mode of action of ochratoxin A carcinogenicity

Carcinogenicity

The carcinogenicity of ochratoxin A in both rats and mice is well established. Ochratoxin A induces renal tumours in rats of both sexes and in male mice. In the rat kidney, tumour induction is seen at a very low dose level (70 µg/kg b.w.). Moreover, in mice ochratoxin A gives rise to liver tumours in both sexes¹⁷.

A correlation between carcinogenicity and exposure to ochratoxin A is not established in humans. However, a correlation has been described between high exposure to ochratoxin A (high level of ochratoxin A in the blood) and high frequency of Balkan Endemic Nephropathy, and it has been found that urinary tract tumours are present with very high incidence in regions affected by Balkan Endemic Nephropathy⁸.

Genotoxicity

Ochratoxin A is negative in conventional mutagenicity tests carried out according to standard protocols, i.e. Ames test and tests for gene mutations and chromosomal aberrations in mammalian cell cultures. However, using different test conditions and/or different endpoints, ochratoxin A is reported to be able to cause DNA-strand breaks *in vitro* and *in vivo*, micronuclei, unscheduled DNA synthesis, sister chromatid exchanges *in vitro*, gene mutations in bacterial cells (modified Ames test) and in NIH/3T3 cell lines^{24 25 26 27 28}.

DNA-adducts

It has to be noted that the covalent binding of chemicals or their reactive metabolites to DNA is generally believed to be a key step in the initiation of carcinogenesis by genotoxic agents. It has been reported that, after administration of ³H labelled ochratoxin A to rats, no radioactivity was found in liver DNA or kidney DNA. From these negative results, covalent binding indexes (CBI) of <0.25 for kidney and of <0.1 for liver DNA were calculated²⁹, which are considered to be of no biological significance.

On the other hand, it has been shown repeatedly that ochratoxin A induces DNA-adducts in kidneys, liver and spleen from mice and rats *in vitro* as well *in vivo*. The highest DNA-adduct levels were found in the target organs (kidney and bladder), being most persistent in the kidney 30 31. In addition ochratoxin A has also been shown to induce DNA-adducts in monkey kidney cells and human bronchial cells *in vitro* 32 33 . However, in all the above mentioned studies, the adducts have been measured by use of ³²P-post-labelling techniques, which cannot give a final proof for ochratoxin A-DNA-adducts.

Therefore, at present, it remains to be established whether the DNA-adducts represent direct, covalent binding of ochratoxin A/ochratoxin A metabolites or represent secondary base changes due to indirect mechanisms. Such mechanisms could include oxidative damage, increased binding of endogenous compounds or tissue injury and sustained hyperplasia 34 35 36 29 37 38 39.

Metabolism and kinetics

The biotransformation of ochratoxin A has not yet been elucidated in detail and the possible contribution of metabolites, especially to genotoxicity, is currently unclear. However, recent studies have shown that, *in vitro*, ochratoxin A is converted into DNA-reactive metabolites 40 33. Experiments have indicated that the toxicity of ochratoxin A is related to its isocoumarin moiety 41.

Studies in rats have shown that ochratoxin A is cleared at a much slower rate from the body than its metabolites 42.

Only one study on one subject is available on the metabolic disposition of ochratoxin A in humans. This study indicated that the half-life of ochratoxin A in humans is comparable with the one in monkeys but is about ten times longer than that seen in rats 43.

5. Conclusion

Ochratoxin A is a mycotoxin which possesses carcinogenic, nephrotoxic, teratogenic, immunotoxic and possibly neurotoxic properties. It has also been linked to nephropathy in humans. Ochratoxin A may have a long half-life in humans.

Ochratoxin A is carcinogenic in rodents. In conventional mutagenicity tests it is negative. However, recent data from *in vitro* and *in vivo* tests using less conventional methods have provided evidence of the genotoxic potential of ochratoxin A.

The Committee is aware that further studies are on-going to elucidate the mechanisms involved in ochratoxin A carcinogenicity.

Estimates of tolerable daily intake by other bodies (see background), based on non-threshold mathematical modelling approaches or a safety factor/threshold approach, have ranged from 1.2 to 14 ng/kg b.w./day.

The Committee notes that the higher figure of 14 ng/kg b.w./day was derived using nephrotoxicity as the endpoint. However there is now an increasing concern about potential genotoxicity of ochratoxin A and its mechanism of action as a carcinogen. Therefore the Committee considers it would be prudent to reduce exposure to ochratoxin A 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 b.w./day which have been estimated by other bodies, e.g. below 5 ng/kg b.w./day.

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