Ochratoxin A - sources of exposure and risk assessment

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Summary

Ochratoxin A (OTA) is known to occur throughout the food chain and has been predominantly found in cereal and products derived from cereals, coffee, beer, wine, dried fruits, spices, cocoa and nuts. Surveys carried out in several countries have demonstrated the presence of OTA in human blood and breast milk, indicating a permanent dietary exposure. Different hazard assessments based on carcinogenicity or nephrotoxicity led to Tolerable Daily Intake (TDI) values in the range of 1.2 - 14 ng OTA/kg b.w..

To estimate the intake of OTA from food, studies on the assessment of dietary intake of OTA were carried out in the recent years in European member states such as Germany and also for the entire European population. Twelve countries provided data on the occurrence of OTA in food products, on consumption of these food products and on occurrence of OTA in human blood and human milk.

The exposure of the European consumers did not generally give rise to major health concerns. Evaluation of the dietary intakes revealed differences between countries and diets but showed that the intakes are quite below the TDI of 5 ng OTA/kg b.w./day as suggested by the EU. Higher intakes approaching the TDI were noted for specific consumer groups, particular children, infants and babies. Public health measures therefore should focus on these groups of the population. The EU has set maximum limits for OTA of 5 µg/kg in raw cereal grains including rice and buckwheat, 3 µg/kg for derived cereal products or cereal grains for direct human consumption, and 10 µg/kg in dried vine fruits. Limits for other products are being considered.

As most of the surveys represent the European situation and diet, it is difficult to transfer these data to other countries of the world. Thus, more surveys are needed in regions of the world other than Europe in order that intake in those regions may be assessed.

Key words: mycotoxin, food contamination, human exposure, mycotoxin legislation

Introduction

Ochratoxin A (7-L-β-phenylalanylcarbonyl-5-chloro-8-hydroxy-3,4-dihydro-3-R-methylisocoumarin) is a nephrotoxic and nephrocarcinogenic mycotoxin produced by
Penicillium verrucosum and P. nordicum in temperate or cold climates and a number of species of Aspergillus in warmer and tropical parts of the world. The mycotoxin is classified by the International Agency for Cancer Research (IARC) as a possible human carcinogen (group 2B), based on sufficient evidence for carcinogenicity in animal studies and inadequate evidence in humans.

Ochratoxin A was discovered in 1965 by van der Merwe et al. and was first encountered as a natural contaminant in maize. OTA was found to be a major causal determinant of the mycotoxic porcine nephropathy and has been found since its first discovery as contaminant in a variety of foods and feeds. Human exposure has been revealed in several European countries based on the detection of OTA in the blood and milk, and the toxin is discussed to play a role in the pathogenesis of BEN, the Balkan Endemic Nephropathy.

In the recent years the importance of OTA was more and more reflected by the work on risk assessment by different organisations such as the Codex Alimentarius Commission and the Scientific Committee on Food of the European Commission.

In this paper the results on the assessment of dietary intake of OTA by the population of EU member states including data from a German ochratoxin A project will be summarized.

**Sources of exposure**

OTA has been found to occur predominantly in cereals and cereal products but also in a variety of other food commodities and even coffee and beer. By far, most data on the natural occurrence of OTA were obtained from Europe. The data on OTA over a five years period document that about 85% of the data originated from Europe.

Fig. 1. Origin of data for 23,167 samples of food analysed for natural occurrence of OTA.
(Croatia, Denmark, Finland, France, Germany, Italy, The Netherlands, Norway, Spain, Sweden, Switzerland, and the United Kingdom), 7% from South America (Brazil and Uruguay), 6% from North America (Canada and the USA), 1% from Africa (Sierra Leone and Tunisia), and 1% from Asia (United Arab Emirates and Japan)\(^7\).

Analyses of human serum samples in Japan, Canada and several European countries revealed that blood from healthy humans frequently contains ochratoxin A\(^7,9\), which indicates a continuous and widespread exposure. Moreover, breast milk represents a relevant source of exposure for lactating babies\(^10\).

Ochratoxin has a half-life of about 35 days in humans, and the blood concentrations are considered to represent a convenient biomarker of exposure during recent weeks\(^7,9\).

Besides the dietary intake of OTA, the toxin has also been found in high concentrations in mycophagic mites\(^11\), cereal dust samples and blood samples of malt factory workers which point to an inhalation exposure of OTA\(^12\).

An overview on the different sources of exposure, among which food of vegetable origin is the most important, is given in Fig. 2.

![Fig. 2. Overview on sources of ochratoxin A exposure.](image)

**Risk assessment**

The factors which play a role in the decision-making process required for mycotoxin legislation and setting maximum levels include scientific, economic and political aspects.

Risk assessment as part of the scientific aspects is based on the toxicological data of OTA (hazard assessment) and the calculation of the acceptable or tolerable daily intakes (ADI or TDI-values), the occurrence data and finally the calculation of the dietary intake using consumption data (exposure assessment) (Fig. 3). As the uptake varies
within different consumer groups special attention must be paid to babies or infants, who may be susceptible to smaller amounts of contaminants.

- Toxicological data (hazard assessment)
  - Identification of toxic compounds
  - Metabolism
  - Acute and chronic toxicities (animal experiments)

- Occurrence data (exposure assessment)

- Nutritional behaviour, food consumption data

**Fig. 3. Steps of risk assessment.**

For OTA different tolerable or acceptable daily intakes (TDI or ADI) have been calculated. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has evaluated OTA in 1991 and 1995. The assessment is based on the nephrotoxic effect in pigs as the most sensitive species with a Lowest Observed Adverse Effect Level (LOAEL) of 8 μg/kg body weight. By use of a safety level of 500, JECFA in 1991 arrived at a TDI of 16 ng/kg b.w., which was converted to a Provisional Tolerable Weekly Intake (PTWI) of 112 ng/kg b.w.. Four years later, in 1995, this value was rounded off to 100 ng/kg b.w. without changing the toxicological evaluation. A further review is planned from JECFA for 2004.

The Canadian authorities have evaluated OTA based on carcinogenic properties in 1989, 1990, 1991 and 1996 and suggested a Provisional TDI of 1.2 - 5.7 ng/kg b.w. A highest tolerable intake of 5 ng OTA/kg b.w. was proposed in 1991 by a Nordic expert group on food toxicology which made an assessment based on carcinogenicity. The European Scientific Committee on Food (SCF) expressed an increasing concern about the potential genotoxicity of OTA and its mechanism of action as carcinogen. Therefore the Committee considered it would be prudent to reduce exposure to OTA as much as possible, ensuring that exposures are at the lower end of the range of tolerable intakes of 1.2-14 ng/kg b.w., e.g. below 5 ng/kg b.w./day.

**German ochratoxin A—Study**

To determine the contamination of food with ochratoxin A (OTA) and the exposure of the German consumer to this mycotoxin, a study was carried out during 1995 and 1999. The data obtained in this study are part of the report on the assessment of dietary intake of Ochratoxin A by the population of the EU Member States (SCOOP task
and the paper on safety evaluation of certain mycotoxins in food prepared by JECFA 2001.7)

In the German study three different data bases (OTA levels in blood, OTA levels in foods, consumption data) were established. From these the exposure of the consumer from food items or food groups was determined and finally the daily intakes calculated and compared with the TDI-values. Almost 6,500 food samples categorized into 29 food groups with 137 sub-groups were examined for OTA and the consumption data of more than 2500 persons were recorded by trained interviewers using questionnaires elaborated by nutritional scientists. In addition, more than 1000 human blood samples were analysed for OTA.

The toxin was found to be present in 57.2 % of all food samples examined (Fig. 4). However, only in 7.3 % of the samples, the concentrations detected were above 0.5 µg/kg, in 1.0 % above 3 µg/kg and in 0.5 % above 5 µg/kg.

Apart from cereals and cereal products, coffee and beer also vegetable/fruit juices, wine, sweets/chips, cocoa and dried vine fruits may contribute to OTA intakes by the consumer. OTA is also detectable in some pork products such as kidney and liver, as well as liver sausage and black pudding. Poultry, beef and milk are practically free of OTA. Milk products may be contaminated only by OTA-containing additives such as spices, muesli or dried fruit.

The toxin was very frequently detected in the blood sera (98 % positives), although mostly in very low concentrations (median 0.2 ng/ml). These plasma levels are very similar to those found in a Swedish study.20)

For adults in Germany, levels in the range of 0.5 to 0.6 ng/kg body weight were established on the basis of the mean-case variant (50th percentile OTA concentration x

Fig. 4. Occurrence of ochratoxin A in German foods (N=6,476).
mean plausible portion sizes of consumption).

The level of OTA intake per kg body weight by consumers with a mostly vegetarian diet did not differ from that of the population. Within this population, no sex- or age-specific differences were found for OTA intake per kg body weight. Statistical analyses showed, however, a significant effect due to location in Germany in connection with a higher intake of certain foods. Among the food groups the intake of OTA is mainly due to cereals and cereal products, coffee and beer.

For children, in particular in the younger age groups, the OTA intake per kg body weight was distinctly higher than for adults. The mean daily intake by the 574 children examined in the age group of 4 to 13 years was between 0.7 and 1.2 ng/kg body weight for the mean-case variant. Above all in the age group of 4 to 9 years, OTA intake was more than 1 ng/kg body weight for the mean-case variant. The intakes calculated by the occurrence of OTA in foods and the consumption data are in accordance with the intake estimates based on the investigations of serum levels of OTA. Similar results of these two different approaches to calculate the intake were reported from Sweden\textsuperscript{20}.

The provisional tolerable daily intake (PTDI) estimated by the EU Scientific Committee for Food in 1998 should be below 5 ng/kg b.w.. The results of this study show that, in view of the foods available on the German market at the present time and the food habits of the German population, the daily OTA intake of the consumer in Germany, established by using two different methods, is 0.5 ng/kg body weight. Thus, a percentage of between 9% and 31% of the PTDI levels is covered. The complete results and data of the OTA-project are published in volume 50 of the Journal Archiv für Lebensmittelhygiene\textsuperscript{21-28}.

**European SCOOP task 3.2.7**

Scientific cooperation (SCOOP) tasks involve coordination amongst Member States to provide pooled data from across the EU on particular issues of concern regarding food safety. These data are used to assist the Commission in developing EU legislation to increase protection of consumers. In January 2002 the SCOOP-Report on Ochratoxin A “Assessment of dietary intake of ochratoxin A by the Population of EU Member States” was completed and published\textsuperscript{19}. Between 1999 and 2001 a total of N=18,599 occurrence data from twelve countries (Denmark, France, Finland, Greece, Germany, Italy, Norway, Portugal, Spain, Sweden, The Netherlands, United Kingdom) were collected together with consumption data and the dietary intakes calculated. For that purpose the mean food consumption (arithmetic mean consumption g/person/day) and mean occurrence data (arithmetic mean value of all samples, both positive and negative; in case of negative samples 1/2 values of limits of detection or 1/6 of limits of quantification were considered) were used.
The number of positive samples was found to be 48.8%. The weighted means for the different food items were: 0.27 μg/kg (wheat), 0.165 μg/kg (corn), 0.19 μg/kg (oats), 0.597 μg/kg (rye), 0.301 μg/kg (barley), 0.136 μg/kg (millet), 0.271 μg/kg (rice), 1.62 μg/kg (green coffee), 0.724 μg/kg (processed coffee), 0.02 μg/kg (beer), 0.375 μg/kg (wine), 0.236 μg/kg (cocoa derived products), 1.15 μg/kg (spices) and 2.298 μg/kg (dried fruits).

Among the individual cereal commodities, rye showed the highest level of contamination, with 50% of positive samples. The percentage of positive samples in processed coffee was higher than in green coffee (46% vs. 36%). The data on dried fruits, with particular reference to vine fruits derived products, indicate these commodities to be highly susceptible to OTA contamination. Among the different spices, nutmeg, paprika, coriander and pepper powder were the most highly and frequently contaminated items.

Additionally, a total of N=2,712 data on the occurrence of OTA in serum and plasma was provided by six countries (Germany, Italy, Norway, Spain, Sweden, UK). The concentration of OTA in individual serum/plasma ranged from 0.11 ng/ml (Germany) to 5.58 ng/ml (Spain) with a weighted mean for the adult European population of 0.34 ng/ml. OTA levels in serum/plasma were used to calculate the estimate daily intake according to around 50% bioavailability of OTA and plasma clearance by considering renal filtration as the only route of elimination. The comparison of the results of the two approaches, i.e. the estimated daily intake from serum/plasma and from occurrence/consumption, showed differences between the particular countries. In some the estimated daily intakes from fluids are lower than those derived from occurrence and consumption data, in others the values are found to be higher.

Moreover, data on human milk (N=324) provided by four countries were included in the report and the mean values of concentration were found to range from 0.01—0.18 ng OTA/ml with a weighted mean of 0.09 ng/ml, approximately indicating an average European level. Even if at quite low levels of contamination, OTA levels in milk samples from Italy were ten fold higher than in other countries. The average calculated intake, based on 600 ml milk consumption (100 ml per suck), ranged from 1.0 ng OTA/kg b.w./day (Norway) to 24.0 ng OTA/kg b.w./day (Italy).

Evaluation of the dietary intakes revealed differences between countries and different diets but showed that the intakes are quite below the TDI of 5 ng/kg b.w./day. Higher intakes were found for specific consumer groups. The contribution of the different food groups to the intake is given in Fig. 5 and shows that cereals and cereal derived products are the main contributors, followed by wine and coffee.
Ochratoxin A legislation in Europe

In Figure 6 an overview of the current legislation on Ochratoxin A in Europe is given. Maximum limits were introduced by the EU for raw cereals (5 μg/kg), cereals intended for human consumption (3 μg/kg) and vine dried fruits (10 μg/kg)\textsuperscript{30}. Maximum levels for Ochratoxin A in other food commodities and beverages are likely to be set, probably by the end of 2003. For the following commodities it was a majority of the Committee that the setting of a maximum level for OTA is appropriate: roasted coffee beans/ground roasted coffee, soluble coffee (instant coffee), wine, wine vinegar, wine and/or grape must based beverages, grape juice and grape must.

Measures currently under discussion also focus on baby foods and processed cereal based foods for infants and young children and ingredients used for the manufacture of these foods as well as on dietary foods for special medical purposes intended specifically for infants and ingredients used for the manufacture of these foods.

Conclusion

In conclusion, the results based of the European studies indicate that the levels of exposure to ochratoxin A do not give rise to any major health concerns. The intake of ochratoxin A in adults in Europe appears to be well below the TDI of 5 ng/kg body
weight. The intake in specific consumer groups, in particular children, infants and babies, however, may approach the TDI. It is, therefore, advisable to set limits as low as reasonably achievable for precautionary consumer protection, taking into account that more stringent guidelines must be fixed for children than for adults.

As most of the data and surveys represent the European situation and diet, it is difficult to transfer these data to other countries of the world in order to carry out an exposure assessment. Thus, more surveys are needed in regions of the world other than Europe in order that intake in those regions may be assessed.

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