codex alimentarius commission

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

WORLD HEALTH ORGANIZATION

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POSITION PAPER ON OCHRATOXIN A

(PREPARED BY SWEDEN)

REQUEST FOR COMMENTS AND INFORMATION

Governments and interested international organizations wishing to submit comments on the following Position Paper on Ochratoxin A are invited to do so **no later than 15 January 1999** as follows: Ms. S.P.J. Hagenstein, Netherlands Codex Contact Point, Ministry of Agriculture, Nature Management and Fisheries, P.O. Box 20401, 2500 EK The Hague, The Netherlands (Telefax: +31 70 378.6141; E-mail: s.p.j.hagenstein@mkg.agro.nl), with a copy to the Chief, Joint FAO/WHO Food Standards Programme, FAO, Via delle Terme di Caracalla, 00100 Rome, Italy.

BACKGROUND

1. The 30th Session of the Codex Committee on Food Additives and Contaminants (CCFAC) accepted the offer of Sweden to prepare an updated version of the position paper on Ochratoxin A for circulation, comment and discussion at the 31st CCFAC (ALINORM 99/12, paras. 76-80).

2. The discussion and position papers on Ochratoxin A presented at the 26th, 27th, 28th and 30th Sessions of the CCFAC (CX/FAC 94/17, CX/FAC 95/15, CX/FAC 95/15 -Add.1, CX/FAC 96/12, and CX/FAC 98/16) by the delegation of Sweden served as the basis for this paper.

3. This paper discusses the following topics: mycology, chemistry and occurrence (paras. 4-7), toxicological evaluations and intake data (paras. 8-19), evaluation of exposure (paras. 20-24) and maximum limits (paras. 25-28). It also contains a number of conclusions and recommendations (paras. 29-30). Detailed occurrence data were presented in CX/FAC 94/17 and CX/FAC 95/15-Add.1.

INTRODUCTION

4. The chemical structure of Ochratoxin A contains a 7-carboxy-5-chloro-8-hydroxy-3,4 dihydro-(3R)-methylisocoumarin linked through the carboxy group to a L-ß-phenylalanine. There are several analogues of Ochratoxin A, such as Ochratoxins B and C, which all are fungal metabolites. However, of all the Ochratoxins, Ochratoxin A is the major compound found as a natural contaminant in plant material.[1]

5. Ochratoxin A was until recently believed to be produced only by *P. verrucosum* Dierckx and members of *Aspergillus* subgenus *Circumdati* section *Circumdati* (formerly the *Aspergillus ochraceus* group). Recent reports indicate that it is also produced by some isolates of *A. niger*, *A. carbonarius* and *A.terreus*. Among the *Aspergilli* only *A. ochraceus* Wilhelm (= *A. alutaceus* Berk. and Curt.) and *A. ostanius* Wehmer (from *Circumdati*) have been reported as having any significance in cereals. *Aspergillus* species have not yet been linked to naturally-occurring Ochratoxin A in cereals in warm climates, but they have been found in coffee beans contaminated with Ochratoxin A. However, a strong correlation between Ochratoxin A in rice and an unidentified *Aspergillus* species has been reported. Ochratoxin A producing cultures of *A. carbonarius* and *A. niger* have recently been isolated from dried vine fruit.[2-4]

6. Stored cereals may be invaded by storage fungi, like *P. verrucosum*, when they are not properly dried or growth may start from locally-moistened areas. Surveys from countries with a temperate climate, where *P. verrucosum* is the most important Ochratoxin A producer, indicate that the problem of Ochratoxin contamination is associated with the post-harvest conditions. There is less information on Ochratoxin production by *Aspergillus* species in warmer climates, e.g. whether the toxin production starts already in the field on the growing plant.[5-7]

7. Physical treatment of grain, such as scouring while cleaning the grain prior to milling, have resulted in a >50% reduction of Ochratoxin A contamination in the resultant wheat flour. Milling seems to have no or only a minor effect on the level of Ochratoxin A and it is only partly destroyed during bread making.[8-9]

TOXICOLOGICAL EVALUATIONS AND INTAKE DATA ON OCHRATOXIN A

8. The International Agency for Research on Cancer (IARC) has classified Ochratoxin A as a possible human carcinogen (group 2B), based on sufficient evidence for carcinogenicity in experimental animal studies and inadequate evidence in humans. Ochratoxin A has been positive in a few genotoxicity assays, including the formation of DNA adducts, but not in the traditional Ames test. Besides the potential genotoxic and carcinogenic properties, Ochratoxin A is also an immunotoxic, nephrotoxic and teratogenic substance. Most of the risk assessments have given greater weight to the carcinogenic properties.[10]

9. JECFA evaluated Ochratoxin A at its 37th meeting and established a Provisional Tolerable Weekly Intake (PTWI) of 112 ng/kg body weight for this mycotoxin (equivalent to 16 ng/kg body weight per day). The assessment was based on the lowest observed adverse effect level for kidney damage in pigs and a safety factor of 500. A no-effect level was not observed. JECFA´s calculation did not address the carcinogenic effects of Ochratoxin A. At its forty-fourth meeting in 1995, JECFA reconfirmed the PTWI established at the thirty-seventh meeting, but rounded it off to 100 ng/kg body weight, corresponding to approx. **14 ng/kg body weight per day.**[11]

10. The Canadian expertise evaluated Ochratoxin A and suggested Provisional Tolerable Daily Intakes (PTDIs) of **1.2 - 5.7 ng/kg body weight** for a risk level of 10^{-5}. Based on the carcinogenic,

genotoxic and other toxic properties known at the time: both a safety factor and model based approaches were used in the extrapolations.[1, 12-13]

11. A Nordic expert group on food toxicology made a similar assessment and proposed a highest TDI of **5 ng/kg body weight**.[14]

12. The European Commission's Scientific Committee for Food (SCF) gave an opinion on Ochratoxin A in January 1995. It concluded that Ochratoxin A is a potent nephrotoxic agent and a carcinogen, that it has genotoxic properties and that, although risk assessments have been based on different toxicological end points, there is broad agreement between the calculated values for acceptable safe levels (see above). The SCF provisionally supported the conclusion that an acceptable safe level of exposure would fall in the range of a few ng/kg body weight per day. In 1998, SCF reviewed its opinion and concluded that 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 to 14 ng/kg bw which have been estimated by other bodies, e.g. below 5 ng/kg bw.[15-16]

13. An updated risk assessment of Ochratoxin A was published in 1996, which concluded that the conservative approach in the risk assessment of Ochratoxin A is most appropriate at the present, with greater weight given to the behaviour of the tumours induced. This conclusion was based on the clearly malignant and aggressive behaviour of the tumours, the high incidence of tumours, the high incidence of metastases, the low levels of Ochratoxin A required to cause tumour development and the often early onset and rapid progression of the tumours in the NTP rat study.[17]

14. Human dietary exposure to Ochratoxin A in parts of Bulgaria, Romania and the former Yugoslavia has been associated with Balkan endemic nephropathy, which is a chronic, progressive disease which may result in death and which predominantly affects women. The evidence for Ochratoxin A being the cause of endemic nephropathy in humans is as yet inconclusive. However, its presence in the environment and in humans, and the extensive epidemiological data, as well as its toxicological characteristics, strongly support the need to further elucidate the possible interrelationship between the disease and Ochratoxin A. Evidence suggesting that elevated exposure to Ochratoxin A is associated with human nephropathies in Algeria and Tunisia has also been published in recent years.[10, 18-19]

15. Investigations of the frequency of occurrence and levels of Ochratoxin A in food and human blood samples indicate that foodstuffs are frequently contaminated with Ochratoxin A. If properly validated, human blood data provide the ultimate evidence that exposure has taken place. These measurements also allow for quantification of exposure at an individual level, which is particularly important when conducting epidemiological studies. Several investigations have been performed recently using the same model for estimating the intake of Ochratoxin A from blood samples.[20]

16. A limited survey of serum samples in Canada indicated a mean exposure of 1.6 ng/kg bw per day. Calculations from the same country, based on food analysis and food consumption data (pork derived products and cereal foods) for young children, indicated a mean exposure of 1.5 ng/kg bw per day. On an individual basis, 18.3% of the serum samples indicated an exposure that exceeded 4.2 ng/kg bw per day.[21]

17. In 1994 the European Commission set up a SCOOP task (Scientific Cooperation Task 3.2.2) to provide the SCF with information on European dietary exposure to Ochratoxin A. Thirteen countries participated in the task. Eight countries gave an estimate of mean dietary intake for an average adult person based on food occurrence and consumption data, and these were in the range from 0.7 to 4.6 ng/kg bw per day, with a mean of means of 1.8 ng/kg bw per day. Five countries gave

an estimate of mean intake based on human blood plasma data, and these were in the range from 0.2 to 2.4 ng/kg bw per day, with a mean of means of 0.9 ng/kg bw per day. The intake estimates based on the two different methods are in the same order of magnitude indicating that the main sources of Ochratoxin A intake are known. The main contributor to the dietary intake of Ochratoxin A seemed to be cereals and cereal products. Other possible contributors to the intake of Ochratoxin A were coffee, beer, pork, products containing pig blood/plasma, pulses and spices. More recently other sources of Ochratoxin A have been identified including wine, grape-juice and vine fruit.[22-25]

18. Ochratoxin A has been detected at high levels in human blood samples collected in nephrology departments in Tunisia from nephropathy patients under dialysis, especially those categorised as having a chronic interstitial nephropathy of unknown aetiology. Both food and blood samples were collected from nephropathy patients and controls. The Ochratoxin A determinations showed very different scales of Ochratoxin A contamination in food and blood ranging from 0.1 to 16.6 µg/kg and 0.1 to 2.3 ng/ml, respectively, in controls and healthy individuals and 0.3 to 46 830 µg/kg for food and 0.7 to 1136 ng/ml for blood in nephropathy patients. The differences between control and nephropathy group were significant for both food and blood ($p<0.005$). The main source of Ochratoxin A in this study seemed to be cereal-based food and dried vegetables and beans.[26]

19. In conclusion, occurrence data and intake measurements reveal that the TDIs may be exceeded by some individuals and that cereals are a very important dietary source of Ochratoxin A. Cereals can act as a dietary source of Ochratoxin A either directly, for example through consumption of bread, or indirectly from products of animal origin via contaminated feed. Furthermore, data on human blood levels of Ochratoxin A indicate geographical differences, which may be connected with poorer post-harvest handling of cereals.

EVALUATION OF EXPOSURE

20 At the 30th session of CCFAC in 1998, Sweden offered to update the position paper (CX/FAC 98/16 in the light of the discussion during the meeting. The use of the horizontal approach of the General Standard for Contaminants and Toxins in Food was supported for this purpose. In Table 1 an attempt has been made to calculate the contribution of all possible sources to the total intake of Ochratoxin A.[27]

21. The intake calculation, shown in Table 1, has been made on European occurrence data and diets. For most of the commodities, except cereals and coffee, only limited data are available and consequently this makes the estimation rough. The consumption data are mostly taken from the GEMS/FOOD regional diets, except for those commodities which, besides cereals, may contribute significantly to the intake. For those products, coffee, red wine and beer, mean values from countries with high consumption rate have been used.[28]

22. The average European consumption of grape-juice is not known, but Swiss data indicate that it is very low, about 5 ml/day. However, its Ochratoxin A content might be of concern since it is consumed by young children. Consequently, a high consumption rate has been assumed, but not incorporated into the total intake except for the figures that are given within brackets.[23]

23. The overall daily intake of Ochratoxin A calculated in Table 1 is 3.5 ng/kg bw. This figure coincides well with the SCOOP assessment, which was in the range 0.7 to 4.6 ng/kg bw. According to the General Standard for Contaminants and Toxins in Foods, MLs shall only be set for those foods in which the contaminant may be found in amounts that are significant for the total exposure of the consumer. In CX/FAC 98/13 it is suggested that a significant amount should contribute to more than

10% of the total dietary exposure. Table 1 indicate that cereals, wine, coffee, and grape-juice should be considered for establishing MLs.

24. Reports on occurrence in wine and grape-juice are very limited and can not form the basis for a proposal for a ML. More data have come available for coffee. In the present calculation, coffee represents 12% of the total intake and 8.6 or 3.6% of the TDI established by the Nordic group or JECFA, respectively. Coffee is, on the other hand, mostly traded as green coffee. It is not possible to suggest ML for Ochratoxin A in green coffee at this stage depending on:

- It is questionable if the intake of Ochratoxin A from coffee corresponds to what the Codex General Standard for Contaminants and Toxins in food regards as "significant".
- The distribution of Ochratoxin A in green coffee is extremely inhomogeneous, which makes it difficult to obtain representative samples.
- There are discrepancies between investigations concerning how much of Ochratoxin A is eliminated during roasting.

MAXIMUM LIMITS FOR OCHRATOXIN A IN CEREALS

25. At the 30th meeting of CCFAC a model for intake calculations was discussed (CX/FAC 98/13). In this model it is recommended that when the distribution of the contaminant in a commodity is not known, it may be assumed that the concentration of the contaminant in 50% of the food is at the ML, with a typical concentration in the remaining at 50% of the ML. The distribution of Ochratoxin A in cereals, as for mycotoxins in general, is very skewed to the left and therefore a more moderate model should be used.

26. In the SCOOP report it can be seen that the median level of Ochratoxin A in all cereal analyses reported, except one, is equal to or less than 0.5 μg/kg. From these data we can assume that 50% of the cereals have an Ochratoxin A concentration between 0 and 0.5 μg/kg. The mean concentration of Ochratoxin A in cereals, including all samples, was estimated to 0.2 to 1.6 by the SCOOP working group.

27. In an attempt to adapt the Codex model for intake calculations of Ochratoxin A, the following model of distribution is suggested:

- 50% of the cereals contain 0.5 μg/kg or less
- 45% of the cereals contain levels between 0.5 μg/kg and the ML
- 5% of the cereals contain levels at the ML

If we assume a ML of 5 μg/kg, which is currently used in some countries, this would result in a theoretical mean level of Ochratoxin A of approximately 1.6 μg/kg according to Table 2. This value is in accordance with the highest estimated mean value for Ochratoxin A in European cereals (see para. 26). According to Table 3 intake calculations of Ochratoxin A, using 1.6 μg/kg as an average Ochratoxin A content, results in a daily intake above the Nordic TDI and below the JECFA TDI for all GEMS/FOOD regional diets. The GEMS/FOOD consumption data are based on Food Balance Sheets (FBS), which are about 15% higher than the actual average food consumption, and the Ochratoxin A values are given for the whole raw agricultural commodity, which also overestimates the intake. Furthermore, taking into account that the model used to predict the distribution of Ochratoxin A in all cereals is still rather conservative, it seems appropriate to establish a maximum limit for Ochratoxin A at 5 μg/kg.

28. Nine countries have specific regulations for Ochratoxin A. Legislative limits range from 5 to 50 µg/kg, although lower limits may apply to infant foods. Limits apply either to some foods, particular cereals, or all food. No barriers to trade have been reported, although existing national regulations or guidelines may lead to such barriers.[29]

CONCLUSIONS AND RECOMMENDATIONS

29. The present Position Paper on Ochratoxin A in Food leads to the following recommendations for consideration at the 31st Session of the CCFAC:

I Based on all the toxicological evidence available to date, levels of Ochratoxin A need to be as low as technologically feasible, taking into account economic and social factors. Since "prevention is better than cure", the ultimate way to protect the consumer from the toxic effect of Ochratoxin A is to encourage and ensure a good agricultural practice by:

(i) revealing the critical points where the fungi start growing and producing Ochratoxin A during agricultural production.

(ii) including quality control programs in agricultural production.

(iii) improving the training of individuals involved at all stages of production.

(iv) supporting research on methods and techniques to prevent fungal contamination in the field and during storage.

II It is recommended that a code of practice be established by Codex for the reduction of Ochratoxin A in cereals.

III It is recommended that a Codex maximum limit for Ochratoxin A be established at a level of **5 mg/kg** for cereals and cereal products.

IV It is recommended that Codex should establish sampling plans and methods of analysis for Ochratoxin A in cereals. There are no evaluated sampling plans existing for Ochratoxin A in cereal grain today. Standardisation of methods for determination of Ochratoxin A in cereals and cereal products is ongoing (European Committee for Standardisation: prEN ISO 15141-1, prEN ISO 15141-2) and methods have been evaluated in interlaboratory studies.

30. In order to evaluate the benefits and possible problems, which may arise from the adoption of these proposals, governments and international organizations are requested to consider the following points concerning the above proposal, in particular:

- from producing countries: the consequences for international trade they anticipate if a Codex ML was established for Ochratoxin A at the level of 5 µg/kg for cereals and cereal products.
- estimates of the possible public health consequences that might arise from the establishment of a ML for Ochratoxin A at a level of 5 µg/kg for cereals and cereal products.
- sampling plans and methods of analysis for Ochratoxin A.

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Table 1: Ochratoxin A (OTA) intake based on European data.

Food	Mean OTA µg/kg	Consumption g/day	Daily OTA intake ng/kg bw*	% of Nordic TDI (5 ng/kg bw)	% of JECFA TDI (14 ng/kg bw)	% of total intake
Cereals	0.5 (22)**	226 (28)	1.9	38	14	54
Red wine	0.19 (24)	171 (30)	0.54	11	3.9	15
Coffee	0.9 (32)	29 (31)	0.43	8.6	3.1	12
Beer	0.07 (22)	234 (22)	0.27	5.4	1.9	7.6
Pork	0.1 (22)	76 (28)	0.13	2.6	0.9	3.7
Raisins	2.8 (25)	2.3 (28)	0.11	2.2	0.8	3.1
Spices	11 (22,33,34)	0.5 (28)	0.09	1.8	0.6	2.6
Poultry	0.03 (22)	53 (28)	0.03	0.6	0.2	0.9
Pulses	0.1 (22,31)	12 (28)	0.02	0.4	0.1	0.6
Grape-juice	1.0 (23,24)	(50)***	(0.8)	(16)	(5.7)	(19)
Total			**3.5**	**70**	**25**	

* *Assumed body weight (bw): 60 kg*
** *Reference, see page 7*
***Consumption not known - proposed high consumption*

Table 2: Mean concentration of Ochratoxin A in cereals with an assumed Maximum Level of 5 µg/kg.

Proportion of samples	Concentration range µg/kg	Mean concentration µg/kg	Contribution to the mean of all samples µg/kg
50%	0-0.5	0.25	0.12
45%	0.5-5	2.75	1.24
5%	5	5	0.25
		Mean of all samples:	**1.6**

Table 3: Ochratoxin A intake from cereals, based on a ML of 5 µg/kg for Ochratoxin A and different regional diets.

Region	Consumption [22] g/day	Assumed mean OTA Content* µg/kg	Daily OTA intake** ng/kg bw	% of TDI Nordic	% of TDI JECFA
Europe	226.3	1.6	6.0	120	43
Middle East	430.8	1.6	11.5	230	82
Far East	452.3	1.6	12.1	242	86
Africa	318.4	1.6	8.5	170	61
Latin America	252.5	1.6	6.7	134	48

**Please see para. 27*
*** Assumed body weight (bw) = 60 kg*