Liquid Chromatographic Determination of Ochratoxin A in Pure and Adulterated Soluble Coffee Using an Immunoaffinity Column Cleanup Procedure

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An immunoaffinity column/HPLC procedure was developed to quantify low levels of ochratoxin A (OTA) in green coffee beans, roasted coffee beans, and soluble (instant) coffee with greater than 80% recoveries. The method was used to survey 116 soluble coffee samples from various countries and different manufacturers and showed contamination levels ranging from "not detectable" to 15.9 μg/kg. The highest levels of OTA were detected among soluble coffees that had been adulterated with coffee husks and/or coffee parchments (mean contamination level: 5.9 μg/kg). By comparison, OTA concentrations in pure soluble coffee samples were significantly lower, with a mean contamination level of 1.1 μg/kg. Although higher than normal figures have been found in some products purchased in East European countries, the results of this survey indicate that pure soluble coffee is not a major source of OTA in the diet, with estimated intakes being well within safety limits.

Keywords: *Ochratoxin A; soluble coffee; analysis; HPLC; adulteration*

INTRODUCTION

Ochratoxin A (OTA) is a nephrotoxic and nephrocarcinogenic mycotoxin produced by several fungal species from *Aspergillus* genus and *Penicillium verrucosum*. It is most commonly found in cereals and cereal products, although a wide range of commodities has been reported as containing the toxin (Pohland et al., 1992).

Natural Occurrence. The natural occurrence of OTA in green coffee beans was first reported by Levi et al. (1974). They analyzed 335 samples of green coffee beans and reported that a total of 22 had amounts of OTA at a level close to the limit of detection of the analytical method employed (approximately 20 μg/kg), the positive samples ranging from 20 to 360 μg/kg. The FDA Mycotoxin Analytical Laboratory in New Orleans analyzed 201 samples of green coffee, and two of these were contaminated by OTA at levels of 96 and 24 μg/kg, respectively (Levi, 1980). A total of 502 commercial green coffees entering the port of Trieste (Italy) were analyzed for OTA. No contaminated samples were found in this study (Levi, 1980). Norton et al. (1982) analyzed 31 green coffee samples and found nine samples contaminated with OTA concentrations ranging from <10 up to 200 μg/kg. OTA was also found in nine out of 40 commercial samples of green coffee at a level of 0.5−23 μg/kg (Cantafora et al., 1983), and Tsubouchi et al. (1984) reported this toxin at a level of 9.9−46.0 μg/kg in four of 22 samples of imported green coffee beans. More recently, Micco et al. (1989) found OTA concentrations of between 0.2 and 15.0 μg/kg in 17 out of 29 green coffee samples analyzed, while Studer-Rohr et al. (1994a,b) detected the toxin in 13 of 25 samples of green coffee beans, at levels ranging from 1.2 to 56.0 μg/kg. The data are summarized in Table 1.

In 1988, as a result of the introduction of a newly established HPLC method for the determination of OTA in coffee beans and coffee products (Terada et al., 1986), the presence of OTA in commercial roasted coffee beans was reported for the first time in five out of 68 samples at a level of 3.2−17.0 μg/kg (Tsubouchi et al., 1988). In 1994, Studer-Rohr et al. (1994a,b) reported that OTA was detected in 16 of 40 analyzed coffee brews (1.0−7.8 μg/kg) prepared using roasted coffee samples purchased in various Swiss retail shops and stated that more than 25−50% of the OTA detected in green coffee is, after roasting, eluted into the brew. Another recent survey conducted by the Ministry of Agriculture, Fisheries and Foods (MAFF) in the U.K. showed that 64 out of 80 soluble coffee samples contained OTA levels in the range 0.1−8.0 μg/kg, while 17 out of 20 roasted coffee samples contained OTA levels between 0.2 and 2.1 μg/kg (MAFF, 1995a). Finally, a German survey revealed the presence of OTA in 20 out of 30 commercial roasted coffee samples, at levels ranging between 0.3 and 7.5 μg/kg (Koch et al., 1996).

Fate during Processing. Inconsistencies are found in the literature regarding the influence of the roasting and brewing process on the OTA content of coffee. Levi et al. (1974) have shown that experimental roasting under conditions simulating those of the typical roasting operation (20 min at 200 ± 5 °C) destroyed 77−87% of the OTA added to green coffee. Gallaz and Stalder (1976) also examined the effect of experimental roasting and found that normal roasting destroyed 80−90% of the toxin. Cantafora et al. (1983) could not detect any OTA after roasting two green coffee samples naturally contaminated with 3.8 and 23.0 ppb OTA, while Micco et al. (1989) reported a 90−100% reduction of the OTA level following the roasting of two green coffee samples naturally contaminated with 4.0 and 8.6 μg/kg.

On the other hand, Tsubouchi et al. (1987) reported that OTA in artificially contaminated green coffee beans was only slightly reduced (0−12%) by heat treatment at 200 °C for 10−20 min, and almost all of the toxin was infused into the coffee decoction when the roasted

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Table 1. Occurrence of Ochratoxin A in Commercial Green Coffee Beans and Coffee Products

country	commodity	incidence	range of OTA level (μg/kg)	refs
United States	green coffee, hand-cleaned[a]	19/267	20[b]−360	Levi et al. (1974)
	green coffee, commercial	3/68	20[b]−80	Levi et al. (1974)
	green coffee, commercial	2/201	24−96	Levi (1980)
Italy	green coffee, commercial	0/502	nd[c]	Levi (1980)
U.K.	green coffee, commercial	9/31	<10−200	Norton et al. (1982)
Italy	green coffee, commercial	9/40	0.5−23.0	Cantafora et al. (1983)
Japan	green coffee, commercial	4/22	9.9−46.0	Tsubouchi et al. (1984)
	roasted coffee, commercial	5/68	3.2−17.0	Tsubouchi et al. (1988)
Italy	green coffee, commercial	17/29	0.2−15.0	Micco et al. (1989)
Switzerland	green coffee, commercial	13/25	1.2−56.0	Studer-Rohr et al. (1994a,b)
	coffee brew	16/40	1.0−7.8	Studer-Rohr et al. (1994a,b)
U.K.	soluble coffee, commercial	64/80	0.1−8.0	MAFF (1995a)
	roasted coffee, commercial	17/20	0.2−2.1	MAFF (1995a)
Germany	roasted coffee, commercial	20/30	0.3−7.5	Koch et al. (1996)

[a] From moldy lots. [b] Corresponds to the limit of detection of the method. [c] Not detected.

Table 2. Fate of Ochratoxin A during Coffee Processing

type of contamination	number of samples	OTA content (μg/kg)	process	% reduction	refs
artificial[a]	4	210−350	roasting, 198−210 °C, 5−20 min	77−87	Levi et al. (1974)
artificial	3	80	roasting	90	Gallaz and Stalder (1976)
inoculation[b]	2	17, 43	roasting	80−90	Gallaz and Stalder (1976)
natural	2	3.8, 23	roasting	90−100	Cantafora et al. (1983)
inoculation	4	170−138000	roasting, 200 °C, 10−20 min	0−12	Tsubouchi et al. (1987)
inoculation	3	1260−137000	brewing	0−2	Tsubouchi et al. (1987)
natural	1	6	decaffeination	60	Micco et al. (1989)
natural	2	4.0, 8.6	roasting, 5−6 min	90−100	Micco et al. (1989)
artificial	3	42−47	roasting, 5−6 min	48−87	Micco et al. (1989)
artificial	1	6	brewing	100	Micco et al. (1989)
natural	3	400−1500	roasting, 252 °C, 100−190 s	14−62	Studer-Rohr et al. (1994a,b)
inoculation	2	90−140	roasting, 252 °C, 100−190 s	2−29	Studer-Rohr et al. (1994a,b)
natural	3	4−17	roasting and brewing	50−75	Studer-Rohr et al. (1994a,b)

[a] Coffee beans were spiked with OTA standard solution. [b] Coffee beans were inoculated with a spore suspension of *Aspergillus ochraceus* and incubated.

samples were ground and extracted with boiling water. This is broadly in agreement with results published by Studer-Rohr et al. (1994a,b) but in contradiction with those of Micco et al. (1989), who examined the OTA content in beverages prepared from artificially contaminated coffee and found no residues of OTA (which is not so surprising since in their experiments the OTA concentration was low in the green beans and 90−100% was destroyed by the roasting process).

It has been suggested (Tsubouchi et al., 1987) that the discrepancy between these findings may be due to differences in the method of introducing the toxin into the ground beans. Indeed, Levi et al. (1974) spiked the ground beans with OTA, while in the experiments conducted by Tsubouchi et al. (1987), the toxin was produced by inoculating whole green beans with toxigenic *Aspergillus ochraceus*. The toxin produced in this manner may be more strongly heat-resistant as a result of the binding of OTA to components of the beans and may more accurately represent the natural contamination of coffee beans. However, this is not clear on examination of the data summarized in Table 2; Gallaz and Stalder (1976) observed no significant difference between the two methods of contaminating the coffee beans, while Micco et al. (1989) even found a more efficient OTA destruction in inoculated beans versus artificially contaminated samples.

Another possible reason for inconsistencies is that analytical methods employed until recently would not have been sufficiently sensitive and selective to detect low levels of OTA in roasted coffee beans and coffee decoctions. Indeed, the analysis of coffee products presents a high degree of difficulty owing to acidic substances extracted together with OTA. As a consequence, methods of analysis for OTA in coffee and coffee products have traditionally involved complex and time-consuming solvent cleanup procedures followed by quantitation using thin-layer chromatography (Levi et al., 1974; Gallaz and Stalder, 1976) or high-performance liquid chromatography (Cantafora et al., 1983; Terada et al., 1986; Studer-Rohr et al., 1995). Recently, a major improvement was introduced by Nakajima et al. (1990), who were the first to use immunoaffinity chromatography for the analysis of OTA in coffee products. They developed their own monoclonal antibody affinity columns and proposed ion-pair chromatography for the final OTA quantitation.

In this study, we evaluated the applicability of commercially available immunoaffinity columns for cleanup and analyzed the OTA eluate by conventional reversed-phase HPLC with fluorescence detection. This method, adapted from that published by Nakajima et al. (1990), proved extremely useful for the rapid cleanup of OTA not only in soluble coffee but also in green and roasted coffee beans. Moreover, it gives higher recoveries and significantly cleaner HPLC chromatograms as well as a lower limit of detection than those reported in previous studies.

MATERIALS AND METHODS

HPLC Equipment. The HPLC system consisted of one Model 420 dual piston pump, a Model 460 autosampler, a SFM 25 fluorescence detector, and a data control system 450, all from Kontron Instruments AG (Zürich, Switzerland).

Chemicals and Reagents. Ochratoxin A was obtained from Sigma Chemical Co. (St. Louis, MO). Methanol, chloroform, toluene, glacial acetic acid, sodium acetate trihydrate,

and sodium hydrogen carbonate were obtained from Merck (Darmstadt, Germany) and were of analytical grade. Acetonitrile was of HPLC grade (Lichrosolv, Merck). Boron trifluoride−methanol complex (10% solution in methanol) was obtained from Fluka (Buchs, Switzerland). Distilled water was withdrawn from a Millipore Milli-Q instrument (Milford, MA). Phosphate-buffered saline (PBS) was prepared using PBS tablets from Oxoid (Basingstoke, U.K.).

The Ochratest immunoaffinity columns were purchased from Vicam Inc. (Watertown, MA). These columns contain an anti-OTA antibody immobilized onto a gel material and are filled with preserved buffer solution. They can be stored for 1 year at room temperature (below 30 °C) without loss of performance. The specifications for these columns are that they will recover >90% when 150 ng of OTA standard is spiked into 10 mL of a toxin-free diluted and filtered corn extract prepared according to the Ochratest procedure.

Standard Preparation. A stock solution of OTA at 20 μg/mL in toluene/acetic acid (99:1) was prepared and assayed according to AOAC Method 973.37 (Stoloff and Scott, 1995; Hald et al., 1993). The stock solution was diluted to 0.02, 0.01, and 0.005 μg/mL in HPLC mobile phase before analysis. For spiking the samples, an aliquot of stock solution was evaporated to dryness and redissolved in the HPLC mobile phase to give a final concentration of 0.1 μg/mL.

Sample Preparation. Finely ground coffee beans (25 g) or soluble coffee (5 g) was weighed accurately into plastic centrifuge bottles and mixed with respectively 500 or 100 mL of methanol−3% aqueous sodium hydrogen carbonate (50:50). The suspension was blended for 3 min at medium speed using a Polytron homogenizer (Ultra-Turrax T25, Janke & Kunkel, Germany). This homogenized sample was filtered through a 5.5 cm Whatman GF/B glass microfiber filter under reduced pressure. Then 4 mL of filtrate was transferred to a graduated cylinder and diluted to 100 mL with PBS.

Affinity Cleanup. The whole diluted extract was applied to an immunoaffinity column, at a slow steady flow rate of 2−3 mL/min. After washing the column with 10 mL of distilled water, OTA was eluted with 4 mL of methanol. To ensure complete removal of the bound toxin, the methanol was left in contact with the column for at least 3 min. This was achieved by reversing the flow of methanol (backflushing) two or three times. The eluate was then evaporated to dryness under a stream of nitrogen at 40 °C, and the residue was redissolved in 150 μL of HPLC mobile phase.

HPLC. A Spherisorb ODS II column (5 μm particle size, 250 mm × 4.6 mm i.d.) with an ODS Hypersil guard column (5 μm particle size, 25 mm × 4.6 mm i.d.), both from Metrohm Bischoff AG (Leonberg, Germany), were used along with a mobile phase of 45% acetonitrile−55% 4 mM sodium acetate/acetic acid (19:1). The separation was performed at ambient temperature at a flow rate of 1.0 mL/min. The injection volume was 75 μL for both standard solutions and sample extracts. The fluorescence detector was operated at an excitation wavelength of 330 nm and an emission wavelength of 470 nm. OTA concentrations in coffee extracts were determined from the standard curve, using peak area for quantitation. If sample measurement was not within points on the standard curve, the sample extract was diluted quantitatively with the HPLC mobile phase and reinjected.

Confirmation of Identity by Methyl Ester Formation. The remaining sample extract was transferred to a 2 mL reactivial and evaporated to dryness under a stream of nitrogen at 40 °C, and the residue was redissolved in 300 μL of boron trifluoride−methanol complex (10% solution in methanol). The solution was mixed thoroughly, heated for 10 min in an oven at 80 °C, and allowed to cool to room temperature. Then 75 μL of derivatized extract was injected into the HPLC system under conditions as specified above. A positive confirmation of identity was provided by disappearance of the OTA peak at a retention time of ca. 14 min and appearance of a new peak (OTA methyl ester) at a retention time of ca. 37 min.

RESULTS AND DISCUSSION

Sample Preparation and Chromatographic Conditions. The proposed method is based on a procedure originally described by Nakajima et al. (1990) but uses commercially available immunoaffinity columns, a different extraction technique, and a different solvent for the elution of OTA from the columns, as well as improved HPLC conditions. First, we observed that the extraction of OTA with a mixture of methanol−3% aqueous sodium hydrogen carbonate (50:50) followed by high-speed homogenization as suggested respectively by Koch et al. (1996) and Tsubouchi et al. (1987) yielded higher OTA levels (by 15−25%) compared to those obtained with a 1% aqueous sodium hydrogen carbonate solution. Initial results also showed that elution of OTA from immunoaffinity columns using 50% dimethyl sulfoxide as recommended by Nakajima et al. (1990) was not effective in removing OTA from commercial Ochratest columns, which may be due to differences in the antibody or in the composition of the packing material (gel). Much better results were obtained by using pure methanol for the quantitative elution of bound OTA. Initially, HPLC separations were carried out using a mobile phase composition of 45% acetonitrile−55% water/acetic acid (41:2), as proposed by Baumann and Zimmerli (1988) for the determination of OTA in various food commodities. However, significant changes in the OTA retention time were observed from day to day with freshly prepared mobile phase, even when taking extra care to minimize batch-to-batch variations. The problem was solved by buffering the HPLC mobile phase, and the optimum composition eventually turned out to be 45% acetonitrile−55% 4 mM sodium acetate/acetic acid (19:1).

Typical HPLC chromatograms obtained for the analysis of soluble coffee using the immunoaffinity approach are shown in Figure 1. The OTA peak is well resolved from the early eluting matrix peak and from other minor components generally present in coffee extracts, which was often not the case with previously published methods (Cantafora et al., 1983; Terada et al., 1986; Tsubouchi et al., 1988) where OTA peaks appeared as shoulders on matrix peaks or were even completely obscured by background peaks in some chromatograms. This illustrates the high selectivity of the immunoaffinity cleanup and thus the ability to achieve a low limit of detection.

Recovery Experiments. The results of recovery experiments on green coffee beans, roasted coffee beans, and soluble coffee spiked with OTA are summarized in Table 3, which also shows the precision achieved. Where relevant, the recoveries were corrected for any natural contamination as indicated by the analysis of the unspiked material. Recoveries from spiked materials with OTA concentrations ranging from 0.5 to 5.0 μg/kg fluctuated between 90% and 108% for green coffee beans (mean 99%) and between 89% and 100% for roasted coffee beans (mean 93%). For soluble coffee, recoveries ranged from 80% to 103% (mean 92%) with relative standard deviations (RSD) between 3.5% and 12.4%. This compares favorably with a mean recovery of 103% (RSD 4.4%) obtained by Nakajima et al. (1990) for soluble coffee powder spiked at the 20 μg/kg level. Contrary to observations made by Koch et al. (1996), no difference was observed in our experiments between recoveries obtained on regular or decaffeinated soluble coffee samples. The detection limit of the method was found to be 0.2 μg/kg, which is comparable to that

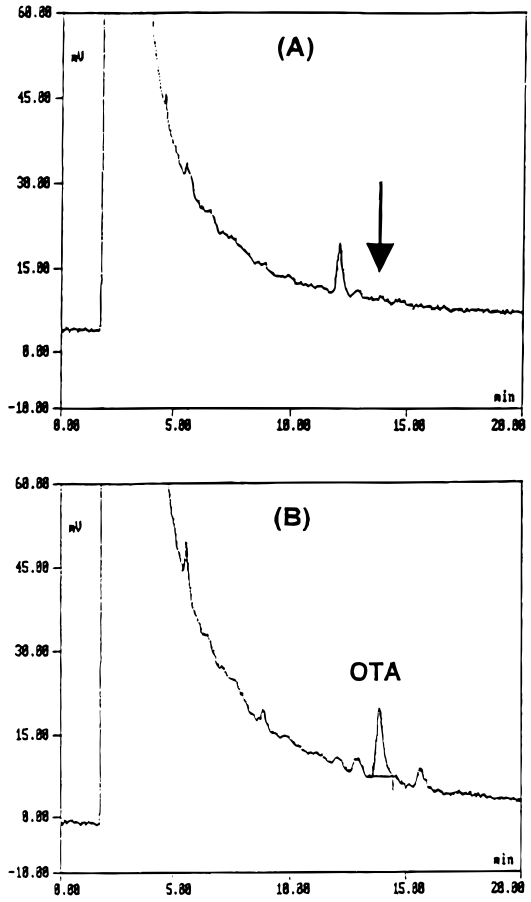

Figure 1. HPLC chromatograms illustrating the analysis of ochratoxin A (conditions given in text): (A) soluble coffee extract, OTA not detectable at a 0.2 μg/kg limit, arrow indicates retention time of OTA; (B) soluble coffee extract, naturally contaminated with 1.6 μg/kg OTA.

Table 3. Recovery of Ochratoxin A Added to Coffee Products

sample	OTA concn measured in blank (μg/kg)	OTA added (μg/kg)	recovery[a] (%)	RSD (%)
green coffee beans	0.8	0.5	108	4.8
		1.0	97	10.1
		2.0	99	13.0
		5.0	90	5.2
roasted coffee beans	not detected	0.5	100	9.9
		1.0	89	14.3
		2.0	90	3.8
		5.0	91	2.7
soluble coffee	0.3	0.5	103	3.5
		1.0	84	12.4
		2.0	80	4.6
		5.0	101	5.4

[a] Average of five determinations on separate extracts. Where relevant, recoveries were corrected for the OTA level found in the blank sample.

reported by Nakajima et al. (1990) and sufficiently low for quality control purposes.

Analysis of Commercial Soluble Coffee Samples. Results of the analysis of 101 pure soluble coffee samples from various countries and different manufacturers are shown in Table 4. OTA was detected at levels ranging from 0.2 to 6.5 μg/kg in a high percentage of samples (75%), which is comparable to the range and incidence reported by the Ministry of Agriculture, Fisheries and Foods in their recent survey of U.K. retail coffee products (MAFF, 1995a). OTA was not detected in 26 samples (i.e., samples contained <0.2 μg/kg), and

Table 4. Levels of Ochratoxin A Found in Pure Soluble Coffee Samples

country of sale	frequency of OTA occurrence	range of OTA concns (μg/kg)	mean OTA level[a] (μg/kg) lower bound value	mean OTA level[a] (μg/kg) upper bound value
Australia	7/22	0.2−4.0	0.3	0.5
Czech Republic	2/2	1.9−4.8	3.4	3.4
Germany	5/9	0.3−2.2	0.6	0.7
Greece	6/6	0.5−1.6	0.7	0.7
Hungary	14/14	0.5−6.5	2.1	2.1
Russia	21/22	0.2−3.5	0.8	0.8
Salvador	6/6	0.3−3.6	1.4	1.4
Slovakia	4/4	1.5−5.3	3.7	3.7
Switzerland	2/3	0.2−0.3	0.2	0.2
Thailand	3/3	1.3−1.9	1.5	1.5
United States	3/6	1.5−2.1	1.0	1.1
miscellaneous	2/4	0.3−0.4	0.2	0.3
overall	75/101	0.2−6.5	1.0	1.1

[a] Limit of detection (LOD) = 0.2 μg/kg. The lower bound mean values were calculated by assuming that samples < LOD contained OTA at 0 μg/kg. The upper bound mean values were calculated by assuming that samples < LOD contained OTA at the LOD level of 0.2 μg/kg.

a total of 41 samples contained OTA at levels ranging from 0.2 to 1.0 μg/kg. A further 18 samples were found to contain more than 2 μg of OTA/kg, among which nine contained more than 3 μg of OTA/kg. Using the MAFF approach, the lower bound mean value (calculated by assuming that samples < 0.2 μg/kg contained OTA at 0 μg/kg) in pure soluble coffee was 1.0 μg/kg, while the upper bound mean value (calculated by assuming that samples < 0.2 μg/kg contained OTA at 0.2 μg/kg) was 1.1 μg/kg. This is nearly identical with figures obtained in the U.K. survey (MAFF, 1995a).

Approximately 50% of the samples collected for this study had been previously analyzed for their total carbohydrate profile according to a method described elsewhere (Prodolliet et al., 1995a). The level of total xylose, which is a good indicator of fraudulent addition of coffee husks or coffee parchments in soluble coffee (Prodolliet et al., 1995a,b), is listed in Table 5 for a separate group of 15 samples (all purchased in East European countries) that were clearly shown to fall into the adulterated category. Indeed, according to statements of the European Soluble Coffee Manufacturer's Association (AFCASOLE), soluble coffee should be considered as adulterated with coffee husks/parchments if the total xylose content exceeds 0.58%, which actually corresponds to the maximum permissible xylose level (0.40%) plus the analytical variability interval (0.18%) (Prodolliet et al., 1995b; MAFF, 1995b). As shown in Table 5, OTA was detected in each of these samples at levels ranging from 1.2 up to 15.9 μg/kg. For the six samples containing OTA levels greater than 5 μg/kg, a confirmation of identity by methyl ester formation was carried out according to a procedure similar to that described by Nesheim et al. (1992). The overall mean OTA content in the 15 adulterated samples was close to 6 μg/kg, which is about 6 times higher than that found in pure soluble coffee samples. This can be explained by the fact that coffee husks are more susceptible to support mold growth and OTA production than the coffee beans themselves and demonstrates that food adulteration is not only an economic issue but may also have an influence on the safety of the incriminated products.

Toxicological Aspects. Ochratoxin A causes a number of adverse effects in laboratory animals includ-

Table 5. Levels of Ochratoxin A Found in Adulterated Soluble Coffee Samples

sample no.	country of sale	total xylose content[a] (%)	mean OTA level[b] (μg/kg)
1	Russia	2.10	15.9
2	Russia	1.22	15.1
3	Russia	1.30	12.7
4	Russia	2.65	9.8
5	Russia	2.78	8.4
6	Russia	1.63	5.5
7	Russia	2.47	4.9
8	Russia	3.35	3.5
9	Russia	4.07	2.0
10	Czech Republic	2.27	2.0
11	Russia	3.60	1.9
12	Russia	4.04	1.9
13	Russia	2.90	1.6
14	Hungary	2.01	1.5
15	Czech Republic	1.00	1.2
overall mean			5.9

[a] Soluble coffee samples are adulterated with coffee husks/parchments if the total xylose content is >0.58%. [b] Average of two determinations.

ing nephrotoxicity, nephro- and hepatocarcinogenicity, teratogenicity, neurodevelopmental toxicity, and immunotoxicity (IARC, 1993). The most notable and sensitive of these toxic effects are nephrotoxicity and nephrocarcinogenicity (Dirheimer and Creppy, 1991). Following an evaluation of the human and animal carcinogenicity data, the International Agency for Research on Cancer (IARC, 1993) recently concluded that there is sufficient evidence in animals for kidney carcinogenicity of OTA but inadequate evidence in humans. The overall evaluation of IARC was "Ochratoxin A is possibly carcinogenic to humans (Group 2B)".

Ochratoxin A has been associated with nephropathy in humans from the Balkan Area (Balkan Endemic Nephropathy, BEN) and with the occurrence of kidney tumors (Krogh, 1974; Plestina, 1992). However, the Joint FAO/WHO Expert Committee on Food Additives (WHO, 1991) concluded that the epidemiological data on BEN and kidney tumors were not conclusive with respect to the role of OTA since other factors might be involved.

On the basis of the kidney toxicity of OTA in pigs, the most sensitive animal species, and using a 500-fold margin of safety, the Joint FAO/WHO Expert Committee on Food Additives (WHO, 1991) has allocated a provisional tolerable weekly intake for OTA of 112 ng/kg of body wt. In 1995, this was rounded down to a value of 100 ng/kg of body wt/week (WHO, 1996). The Nordic Working Group on Food Toxicology and Risk Evaluation (1991) estimated acceptable safe levels for OTA exposure using a series of different risk analysis models whose relevance is dictated by the mechanism of OTA-induced carcinogenesis. The highest tolerable intake of OTA of 5 ng/kg of body wt/day was determined using a safety factor approach, and this was in reasonable accordance with the values (0.4−2.4 ng/kg of body wt/day) calculated for a lifetime risk level for cancer of $1:10^6$ using the Knudson−Mooglavkar model which assumes that OTA does not have initiating properties. This model was considered the most relevant for use in connection with OTA. Kuiper-Goodman (1991), who also used the data from the NTP carcinogenicity study in rats (Boorman, 1989) as the basis for their risk analysis, estimated a tolerable level for OTA of between 1.5 and 5.7 ng/kg of body wt/day depending upon the method of extrapolation employed. Recently the Scientific Committee for Food of the European Commission (1994) provisionally supported the conclusion that an acceptable safe level of daily exposure for OTA would fall in the range of a few nanograms/kilogram of body wt/day.

In conclusion, if it is assumed that soluble coffee is contaminated at an OTA level of 1.1 μg/kg (average concentration found in the 101 pure soluble coffee samples), then one cup of coffee would provide ca. 2.2 ng of OTA (0.03 ng/kg of body wt), which is about 160-fold lower than the acceptable safe daily exposure for OTA. The results of this survey therefore indicate that pure soluble coffee is not a major source of OTA in the diet, with estimated intakes being well within safety limits.

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