Zeitschrift: Mitteilungen aus dem Gebiete der Lebensmitteluntersuchung und Hygiene = Travaux de chimie alimentaire et d'hygiène

Herausgeber: Bundesamt für Gesundheit

Band: 88 (1997)

Heft: 5

Artikel: Mycotoxin contamination of food in Ecuador : B: Ochratoxin A, deoxynivalenol, T-2 toxin and fumonisin

Autor: Mühlemann, Marc / Lüthy, Jürg / Hübner, Philipp

DOI: https://doi.org/10.5169/seals-982338

Mitt. Gebiete Lebensm. Hyg. 88, 593–612 (1997)
Received 11 April 1997. Accepted 12 May 1997

Mycotoxin Contamination of Food in Ecuador*

B: Ochratoxin A, Deoxynivalenol, T-2 Toxin and Fumonisin

Key words: Mycotoxins, Food, Ecuador, ELISA, HPLC

Marc Mühlemann[1], *Jürg Lüthy* and *Philipp Hübner*[2]
Laboratory of Food Chemistry, Department of Chemistry
and Biochemistry, University of Berne

Introduction

Up to date, more than 300 mycotoxins have been identified, produced by approximatively 350 fungal species (1). Within the mycotoxins, aflatoxins (especially AFB_1) are of major concern; they were classified as probable human carcinogens (2). Although the existing limitations on the occurrence of mycotoxins in foods and feeds focus on aflatoxins, contamination levels of other mycotoxins, such as ochratoxin A (OA), the fumonisins B (FB_x), the trichothecenes T-2 toxin and deoxynivalenol (vomitoxin or DON), are also regulated in some countries or the introduction of limits is discussed (3–5).

The toxicological significance and mode of action of these mycotoxins are different: *OA* is produced by several species of the fungal genera *Aspergillus* and *Penicillium.* OA associated nephropathy in farm animals is well documented and recently, the toxin has been detected in the blood of 6 to 18% of the human population in some Balkan areas where endemic nephropathy is prevalent (6). Furthermore, its carcinogenicity was proven in rats (6, 7). *Trichothecenes* all have a basic tetracyclic scirpenol ring system. They are produced primarily by moulds belonging to the genus *Fusarium,* but other genera like *Trichoderma, Trichothecium, Myrothecium* and *Stachybotrys* also produce trichothecene metabolites. The class A group toxins (lack of a ketone group at C-8, e.g.: T-2 toxin) are far more potent than the class B group (e.g.: DON). Trichothecenes are cytotoxic and produce acute systemic effects in various animal species at different doses: epithelionecrosis in the intestinal tract, the lymphoid and haematopoietic tissues by impairment of protein synthesis or by causing disfunction of cellular membranes,

* Part of the thesis of Marc Mühlemann, University of Berne 1996

suppression of cell mediated humoral immunity and decreased resistance to secondary infection by bacteria, yeasts and viruses. Effects in man were historically associated with alimentary toxic aleukia (ATA) in the USSR and scabby wheat toxicoses in Japan and Korea (6). The *fumonisins* are produced by *Fusarium moniliforme* and *F. proliferatum.* They cause equine leucoencephalomalacia (ELEM) and hepatotoxicity in horses; porcine pulmonary edema syndrome (PPE) and hepatocarcinoma in rats (8–10).

In 1991, the state of knowledge about the mycotoxin situation (affected matrices, frequency and extent of contamination) in Ecuador, South America, allowed neither basic estimations of the intake of different mycotoxins nor the performance of any risk evaluation for Ecuadorians (11). The situation concerning aflatoxin contamination levels in Ecuador was recently adressed (12). The aim of the present work is to get a rough estimate on OA, DON, T-2 toxin and FB contamination levels in Ecuadorian foods and feeds in order to recognise existing problems and enabeling a basic risk evaluation.

Materials and methods

Chemicals

OA, DON and T-2 were purchased from Sigma Chemical Company (St. Louis). They were diluted and quantified according to (13). Distilled or deionized water was used throughout. Reagents and solvents (with at least p. a. quality) were purchased from Merck, Romil, Fluka, Sigfried, Riedl-de Haen or Baker. Benzene and toluene (both distilled) and $K_2Cr_2O_7$ were kindly provided by Dr. Espin de Rivera from the Instituto Nacional de Investigacion Agropecuaria (INIAP), Quito. Folded filter papers used were from Schleicher & Schüll, type 597 1/2 for all ELISA analysis and samples for HPLC were filtered over 0.2 µm Acro LC 13 filters from Chromopack.

Sampling

Samples were taken between April 1992 and October 1994 and included rice and subproducts (99 samples), hard, medium hard and soft corn (89 samples) and beans (79 samples).

Mixed samples (1.2 to 3.0 kg) of rice, corn (including corresponding subproducts) and beans were randomly collected from different Ecuadorian climatic regions paying attention to variety, origin, type of storage, marketing and moisture content. For futher details refer to (12).

Extraction of toxins for ELISA analysis

Extractions were performed, with exception of slight modifications, according to the instructions of the producer (Neogen Corp., Lansing MI) supplied with the employed semiquantitative test kits (Agri-Screen for Ochratoxin, DON (Vomitoxin) and T-2 toxin). The same extraction procedures were used for the subsequently produced quantitative Veratox test kit (Neogen Corp., MI). 25 g (DON: 20 g) of pretreated rice, corn and bean samples were mixed with 50 ml of 80% methanolic solution (OA analysis), 200 ml of water (DON analysis) and 125 ml of 70% methanolic solution (T-2 toxin analysis). These mixtures were shaken on a Lab-Line shaker (12 min, 260 rpm) at room temperature and filtered. Contact time between sample and extraction solvent totalled 45 min. The filtrates were applied to ELISA analysis without further purification. The ELISA analyses of toxins were performed according to the producers directions.

Extraction and purification of ochratoxin A for HPLC analysis

The extraction procedure for OA was according to (14) with the modifications described in (12). The basic principle of the purification of OA for HPLC analysis is described by (15). The residues dissolved in benzene/acetonitrile were dried down in a water bath (max. 40 °C) under a gentle stream of nitrogen and quantitatively redissolved in 100 ml chloroform. The chloroform solutions were applied to a preconditioned Extrelut column: 7 g Extrelut filling material was mixed with 10 ml of a 1% $NaHCO_3$ solution. The adsorbens (at pH 9) was applied onto a Extrelut column. The chloroform solution was slowly passed through the column, the column was washed twice with 40 ml chloroform; the washing solutions were discarded. The mixture of 30 ml chloroform with 1 ml 100% formic acid was mixed with the adsorbens. The eluted solution and the subsequent washing solutions (2 x 40 ml chloroform) were collected and evaporated to dryness on a rotary evaporator (Büchi KRvrTD) at max. 40 °C. The residues were quantitatively removed and redissolved in 0.5 ml methanol and stored refrigerated (2–4 °C) until HPLC analysis.

HPLC analysis of ochratoxin A

The basic principle of HPLC analysis of the samples is described in (15). Analysis of the samples was performed with a Kontron 420 system equipped with a heated (50 °C) Spherisorb ODS 1 column, particle size 5 µm, 25 cm x 4.6 mm (Bischoff/Leonberg, art. no: 839540), and a guard column (LiChrosorb RP-18, 30 x 4.6 mm). Detection was by a UV/VIS spectrofluorometer detector (Perkin/Elmer 650-10S), excitation wavelength set at 330 nm and emission wave length at 460 nm. The flow rate was 1 ml/min of mobil phase (methanol/water, 62:38, with 2.5% acetic acid) and the injected volume was 20 µl. The experimental blank value (reagents only) and reference samples without OA contamination from all matrices were

tested for the absence of fluorescent compounds in the interval of OA retention time. Under the described conditions, the retention time of OA was 8.2–8.5 min. OA was quantitated by peak heights comparison between corresponding sample and external standard peak. The identity of OA in high contaminated and spiked samples was confirmed by forming the methyl ester of OA according to (16). After the reaction, the mixtures were dried down under nitrogen (water bath, max. 40 °C), redissolved in 100–400 µl of mobil phase and analysed in the HPLC system mentioned above. The retention time of the methylated compound was 13.2 min.

Artificial contamination of rice, corn and bean samples

In order to determine detection limits and recoveries for ELISA analysis from each matrix, 25 g (DON: 20 g) of samples which tested negative in prior ELISA analysis were spiked with different amounts of toxin per g sample: 10 and 25 ng of OA, 0.2, 0.4, 0.6, 0.75 and 1.5 µg of DON and 50 and 100 ng of T-2 toxin. The solvent was evaporated for 2 h and the extraction procedure followed as described above.

Recoveries of OA from each matrix in HPLC analysis were determined by spiking 70.5 ml of filtered sample extract solution of samples, which tested negative in prior ELISA analysis, with OA at concentrations of 3, 6 and 12 ng toxin/g sample, respectively, in a first set of experiments and 8.54 ng OA/g rice or corn and 4.84 ng OA/g beans, respectively, in a second set of experiments.

Extraction, purification and analysis of fumonisins

The extraction, purification and analysis of fumonisin B_1 and B_2 from 11 corn samples from Ecuador are described in (5).

Statistics

Data below the detection limit were arbitrarily set to half of this value. Statitical analysis of data was performed by analysis of variance (ANOVA) followed by Sheffé's post-hoc test. Significance level was 5%. Error bars in figures represent ± one standard deviation.

Information concerning sampling procedure, transport and pretreatment of samples and determination of moisture content is given in (12).

Results and discussion

Validation of isolation and detection procedures

Recoveries and detection limits for OA and trichothecenes DON and T-2 toxin by ELISA analysis were assessed by spiking experiments in three different matrices

at different concentrations (table 1). The detection limits of *OA* were 25 ng/g in rice and corn, but no statistically significant difference was seen between the controls and bean samples spiked with OA at the indicated levels. Recoveries of artificially

Table 1. Number of analyses with mean value and standard error of recoveries for ELISA-analysis with spiked samples

A: ochratoxin A (OA)						
spiked level (ng/g)	rice		corn		beans	
	number	mean (ng/g) ± std.error	number	mean (ng/g) ± std.error	number	mean (ng/g) ± std.error
0	14	2.5 ± 0.54	14	2 ± 0.62	7	4.5 ± 1.5
10	26	5.5 ± 0.94	26	7.3 ± 1.1	13	5.1 ± 1.3
25	22	9.2[a] ± 2	22	14[a] ± 2.1	11	7.6 ± 2.2

[a] Results shown in bold are statistically significant different to zero-spike (0.0001 ≤ p-value ≤ 0.012).

B: deoxynivalenol (DON)						
spiked level (µg/g)	rice		corn		beans	
	number	mean (µg/g) ± std.error	number	mean (µg/g) ± std.error	number	mean (µg/g) ± std.error
0	6	0.15 ± 0.07	6	0.12 ± 0.05	3	0.03 ± 0.03
0.2	6	0.28 ± 0.1	6	0.22 ± 0.08	3	0.13 ± 0.09
0.4	6	0.48 ± 0.14	6	0.4 ± 0.11	3	0.33 ± 0.19
0.6	6	0.7[b] ± 0.2	6	0.63[b] ± 0.13	3	0.43 ± 0.24
0.75	10	0.93[b] ± 0.05	10	0.75[b] ± 0.07	4	0.6 ± 0.09
1.5	6	1.9[b] ± 0.05	8	1.6[b] ± 0.07	4	1.1[b] ± 0.06

[b] Results shown in bold are statistically significant different to zero-spike (0.0001 ≤ p-value ≤ 0.046).

C: trichothecene T-2 toxin						
spiked level (ng/g)	rice		corn		beans	
	number	mean (ng/g) ± std.error	number	mean (ng/g) ± std.error	number	mean (ng/g) ± std.error
0	8	16 ± 2.7	8	19 ± 5	4	25 ± 6.6
50	12	56[c] ± 3	10	69[c] ± 6.2	4	103[c] ± 9.5
100	10	105[c] ± 4.7	10	104[c] ± 7	5	144[c] ± 4.8

[c] Results shown in bold are statistically significant different to zero-spike (0.0001 ≤ p-value ≤ 0.0056).

added OA reached 56% in corn and 37% in rice. The performance of OA-ELISA analysis of corn was further examined by analysing 11 naturally contaminated corn samples (above the ELISA detection level) by HPLC and subsequent comparison of the two data sets by regression analysis. The regression plot of corn was $OA_{HPLC} = -2.8 + 4.0\ OA_{ELISA}$; $r = 0.88$. Taking into account the low recovery of OA for ELISA analysis, we still find about 50% underestimation of naturally occurring OA by ELISA analysis in corn compared to HPLC analysis. The insufficient over all performance of the OA test kit at the indicated contamination levels could be due to matrix effects, extraction procedure or pH of the samples exceeding the ideal range. AOAC approvement of the OA test kit does not exist. For HPLC analyses recoveries of OA only reached 15–20% in rice, corn and beans in a first set of experiments. After changing the isolation procedures with a second addition of 1 ml 0.33 mol/l phosphoric acid in the first partitioning step recoveries between 61 to 75% were obtained. The detection limit of the HPLC method is reported to be below 0.1 ng OA/g sample (15).

An interlaboratory comparative study was organised in 1995 for Swiss control laboratories practising *trichothecene* analysis (Dr. O. Zoller, Federal Office of Public Health (BAG), Berne). Naturally (at levels of 200–800 ng/g of DON) and artificially (at levels of 2000 ng/g of DON and 500 ng/g of T-2 toxin) contaminated samples of wheat were provided by the BAG. We participated in this study using the ELISA test kits from Neogen Corporation. Eight laboratories analysed the samples with different methods (GC/MS, 4 laboratories; GC/ECD, 1; TLC, 1 and ELISA, 2 laboratories). The general conclusion derived was that the implemented ELISA methods provide results within the current analytical possibilities.

The detection limit of *DON* ranged between 0.6 µg/g (rice, corn) and 1,5 µg/g (beans) sample; recoveries of DON at the detection limit range from 73% (beans) to 117% (rice). These values are in agreement with those reported by the USDA-FGIS approvement for the Veratox vomitoxin (DON) test kit (personal communication G. Boerboom, Neogen Corp., Lansing, MI) and with the recoveries found in the mentioned interlaboratory comparative study. The detection limit of *T-2 toxin* was 50 ng toxin/g sample in all matrices; recoveries of T-2 toxin at the detection limit range between 112% (rice) and 206% (beans) and are improving for higher contamination levels in beans (144% at 100 ng/g). These findings are in agreement with the changed test kit detection limits from the semiquantitative Agri Screen test kit (50 ng toxin/g sample) to the quantitative Veratox test kit (150 ng toxin/g sample) and with the conclusion of the mentioned interlaboratory comparative study. AOAC approvement of these test kits does not exist. Within the interlaboratory comparative study, the mean relative standard deviations among laboratories (RSD's) were 63% for DON and 55% for T-2 toxin (17).

Occurrence of OA, DON, T2 and fumonisin in different Ecuadorian foods

The frequency distributions of the analysed toxins are given in table 2. Most striking are the presence of high values of OA in corn and beans (table 2A) and the occurrence of DON contamination levels above 1 µg/g in all matrices (table 2B).

Table 2. Frequency distribution of mycotoxins in all analysed samples

A1: Ochratoxin A by ELISA							
OA (ng/g) [a]	0–25	–40	–80	–160	total samples	arithmetic mean	median
rice	94	2	3	–	99	14	12.5
corn, soft	32	4	1	1	38	18	12.5
corn, hard	40	2	–	–	42	14	12.5

[a] OA by ELISA text kit «Veratox Ochratoxin quantitative test kit» from Neogen Corp., MI

A2: Ochratoxin A by HPLC, recovery corrected									
OA (ng/g) [b]	0–10	–20	–40	–80	–160	–320	total samples	arithmetic mean	median
rice	16	–	–	–	–	–	16	1.3	0.6
corn, soft	6	–	–	–	1	1	8	42	0.6
corn, hard	4	1	–	–	1	1	7	62	1.8
beans	5	1	3	1	1	–	11	23	10.5

[b] OA by HPLC, adapted method from Baumann and Zimmerli, 1985

B: Trichothecene deoxynivalenol by ELISA								
DON (µg/g) [c]	0–0.6	–1	–2	–4	–8	total samples	arithmetic mean	median
rice	85	10	–	3	1	99	0.48	0.3
corn, soft	44	1	1	–	–	46	0.34	0.3
corn, hard	34	3	2	3	–	42	0.54	0.3
beans	77			–	–	77	0.76	0.75

[c] DON by ELISA test kits «Veratox DON quantitative vomitoxin test» and «Agri-Screen for Vomitoxin (DON) lab test», both from Neogen Corp., MI

C: Trichothecene T-2-Toxin by ELISA						
T-2 (ng/g) [d]	0–50	–80	–160	total samples	arithmetic mean	median
rice	93	5	1	99	27	25
corn, soft	46	1	1	48	27	25
corn, hard	32	9	1	42	35	25
beans	65	11	–	76	30	25

[d] T-2 by ELISA test kits «Agri-Screen for T-2 Toxin lab test» and «Veratox quantitative T-2 toxin test», both from Neogen Corp., MI

D: Fumonisins B_1 and B_2 by HPLC (data from Zoller et al., 1994, recovery corrected)											
FB_X (ng/g)	0–25	–100	–250	–500	–1000	–2000	–4000	–8000	total samples	arithmetic mean	median
FB_1	2	1	–	1	2	2	1	2	11	1865	883
FB_2	3	1	2	4	–	–	1	–	11	386	242

94% of the rice sampels analysed for OA by ELISA tested below the detection limit and the presence of OA contamination levels below 10 ng/g in the corresponding HPLC analysis might be taken as an indication for the absence of high OA contamination in polished rice. The maximal contamination levels of trichothecene T-2 toxin in rice, corn and beans were 94, 123 and 78 ng/g, respectively. Considering the high recoveries of T-2 toxin analysis by ELISA (table 1C), it can be concluded that the contamination with trichothecene T-2 toxin does not pose a major problem to any of the analysed matrices. In addition to this study, high contamination levels of hard corn from Ecuadorian origin with fumonisins (table 2D) were reported (5).

Co-occurrence of the different mentioned toxins in all matrices is possible, but not the rule. The regression blots (each mentioned toxin against each other in each matrix) showed r-values from 0.02 to 0.56. Thus co-occurrence of the different mentioned toxins in a sample is not very probable.

Occurrence of different mycotoxins in different Ecuadorian food

Ochratoxin A

For *rice*, a statistically significant difference ($0.0001 \leq p \leq 0.053$) was found between the polish powder fraction (7 samples, mean: 28 ng/g) and all other rice matrices (5 matrices with mean values between 12.5 and 14 ng/g, totalling 92 samples) in ELISA analysis (fig. 1). HPLC analysis of rice samples revealed contaminations up to 2 ng/g in polished rice (12 samples, mean: 0.8 ng/g) and contaminations up to 9 ng/g in the polish powder fractions (3 samples, mean: 3.4 ng/g) with a p-value of 0.056. The results indicate that OA contamination of rice in the field might occur at low levels, but most of the contamination is separated into the polish powder fraction by polishing and polished rice does not remain contaminated with substantial amounts of OA. Polish powder fractions are used for feeding purpose.

Both *hard and soft corn* proved susceptibility for high OA contamination levels in ELISA and HPLC analysis, which revealed mean and maximal contaminations for soft corn (8 samples) at 42 and 174 ng/g, respectively and for hard corn (7 samples) at 62 and 253 ng/g, respectively. Moreover, a matrix difference ($p < 0.0001$) was observed in soft corn between the early maturing variety used to produce «mote» (2 samples, mean: 162 ng/g) and other in the interandean region produced varieties (6 samples, mean: 2 ng/g). Concerning the appearance of both, hard and soft endosperm corn, no significant difference was seen between optically nice (fresh/brilliant) or normal and mouldy corn (fig. 2A). These results indicate the possibility of high OA contamination levels in nice and mouldy corn without the

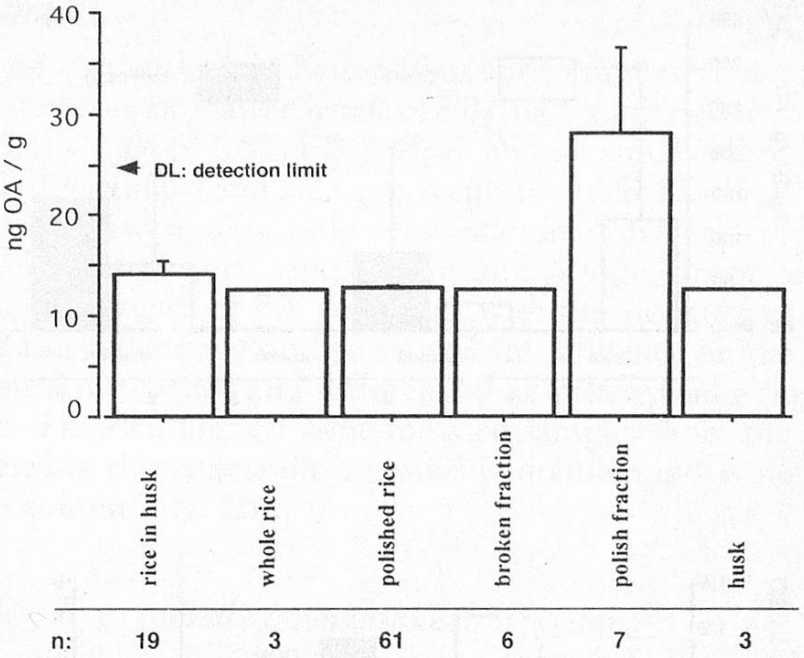

Fig. 1. Influence of matrix on levels of ochratoxin A in naturally contaminated rice, as analyzed by ELISA

opportunity of quality judgement by the appearance. Taking in account the measured DON contents in mountain region grown corn (see below), these results are in agreement with the FAO study (18) describing mountain region grown corn contaminated by the mould species *Fusarium, Penicillium, Nigrospora, Phizoctonia, Aspergillus and Diplodia* (in order of importance of incidence).

11 *bean* samples analysed by HPLC revealed a mean contamination of 23 ng OA/g, whereby the highest contamination level was seen in the variety «Canario». Concerning the appearance, no statistical difference was found between the different classifications; that means, OA contamination in beans is not related to optical quality judgement parameters and the highest contamination level was found in a sample judged to be of nice aspect.

Vomitoxin

In *rice*, no statistically significant difference was seen concerning DON, but rice in husk (19 samples, mean: 0.56 µg/g) showed intermittent contamination levels between the polish powder fraction (7 samples, mean: 0.76 µg/g) or the broken fraction (6 samples, mean: 0.83 µg/g) and all other fractions (whole and polished rice and husk with mean values of contamination levels from 0.3–0.41 µg/g). Thus half ore more of the vomitoxin contamination separates into the polish powder and broken fraction during polishing and DON contaminations in rice seems mainly to be a field problem (11 field samples, mean: 0.76 µg/g) and not a post harvest problem.

In *soft corn*, DON contaminations above the detection limit were found only in visible mouldy corn (11 samples, mean: 0.45 µg/g). The contrary situation was observed in hard corn: mean contamination values of 11 samples judged to be of

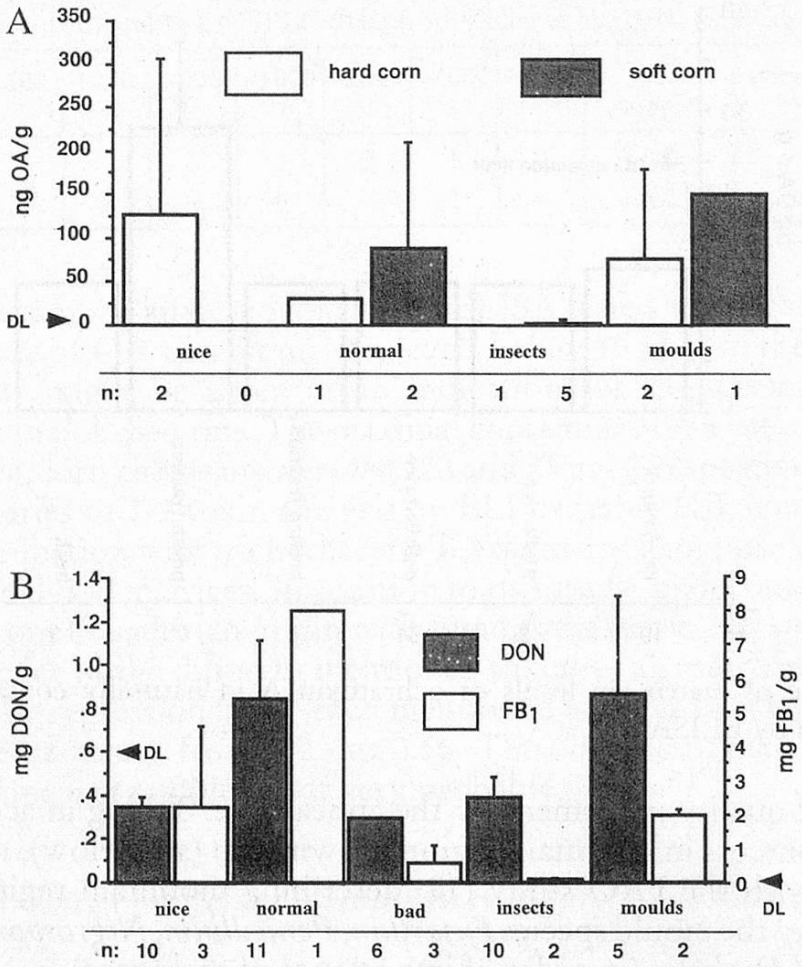

Fig. 2. A = Influence of appearance on ochratoxin A occurrence
B = Influence of appearance on desoxynivalenol and fumonisin B_1 occurrence in hard corn

«normal» aspect were similar to those of 5 visibly mouldy corn samples (0.85 and 0.87 µg/g, respectively, figure 2B). Four samples from the field (mean: 0.88 µg/g) were contaminated above the detection limit and a dependence to moisture content seems to exist ($p = 0.09$). Thus the vomitoxin contaminations in hard corn seem to be partially a field problem.

In *beans* DON contamination values above the detection limit were only found in «Panamito» (16 samples, mean: 0.8 µg/g). The presence of moulds is no indication for any DON contamination.

T-2 toxin

No analysed matrix showed the tendency to contamination of T-2 toxin exceeding 120 ng/g. Thus, in the time period during analysis, T-2 toxin was apparently no problem in any of the analysed matrices.

Fumonisins B_1 and B_2

The fumonisin contents of 11 Ecuadorian corn samples were analysed by (5). Recovery corrected contamination levels of FB_1 (µg/g) correlated with those of FB_2 (FB_1 = 0.46 (± 0.4) + 3.64 (± 0.57) FB_2). Mean and maximal values of FB_1 and FB_2 were 1.9 and 8.0 µg/g and 0.4 and 2.2 µg/g, respectively. From the 11 samples, 3 soft or medium hard corn had significant lower contamination levels (mean: 0.033 µg/g) than the hard endosperm corn samples (mean: 3,1 µg/g) from coast and orient production. The occurrence of FB_1 correlates with the moisture content (r = 0.86; p = 0.0006), and the climate region had significant influence on the contamination level, being highest in the east and different to all other production regions with $0.001 \leq p \leq 0.05$. The two highest contaminated samples from the east were field samples. Concerning the appearance, mould contamination is no indication for fumonisin toxin content (fig. 2B).

Daily toxin intake in Ecuador

For the calculation of the daily intake of the different toxins by Ecuadorians, the mean and median values of toxin contents of the different foodstuffs were used (table 2). For the risk assessment, the mean values of daily consumption of the different food items are needed. From the examined foodstuffs, Ecuadorians eat aproximately 110 g rice, 21 g soft corn and 10 g dried beans (19). The estimated consumption of hard corn is about 10 g per day and person (12); the average weight of Ecuadorians is about 55 kg. Based on arithmetic means, the resulting values of intake by the Ecuadorians (indicated in ng/kg body weight/day) through the different matrices and toxins were 3 (rice), 16 (soft corn), 11 (hard corn) and 4 (beans) for OA; 960 (rice), 130 (soft corn), 100 (hard corn) and 140 (beans) for DON; 54 (rice), 10 (soft corn), 6 (hard corn) and 6 (beans) for T-2 toxin; and 477 (soft and hard corn) for the fumonisins, respectively (table 3).

The total daily intake based on the arithmetic mean values of OA (based on the HPLC measurements), DON, T-2 toxin and fumonisins by Ecuadorians is estimated to be 34, 395, 72 and 1330 ng/kg BW/d, respectively (table 3). Calculations based on the median values led to lower estimates of the daily intake of the mentioned toxins in general and esspecially of OA.

Risk assessment

Ochratoxin A

For comparison, in Canada and central Europe, the daily intake of ochratoxin A is estimated to be ≤ 5 ng/kg BW/day (21–23). Human data reveal the presence of OA usually at levels from 0.2 to 6 ng/ml in the blood of individuals from several European countries (23–26). However, inhabitants of some Balkan regions have milk and blood concentrations of OA up to 100 ng/ml (27). *Krogh* (28) first showed a striking similarity between porcine nephropathy caused by OA exposure and

Table 3. Daily intake of different mycotoxins by the Ecuadorian population based on arithmetic means and (medians) in ng/kg BW per day

Toxin	Toxin intake with different matrices				Total (ng/kg BW/d)
	rice	corn, soft	corn, hard	beans	
Ochratoxin A	3	16[a]	11	4	34
	(1)	(0.2)	(0.3)	(1.9)	(3.4)
Fumonisins $B_1 + B_2$	–[na]	27	450	–[na]	477
		(27)	(260)		(287)
T-2 toxin	54	10	6	6	76[b]
	(50)	(10)	(4.5)	(4.5)	(69)
Vomitoxin	960	130	100	140	1330[b]
	(600)	(115)	(55)	(140)	(910)

values are based on arithmetic means; values in paranthesis are based on medians

[a] value below 4 ng/kg BW/d when calculated excluding soft corn to produce «mote» (2 samples)

[b] according to the interlaboratory comparative study conducted by BAG, Bern, these values may have RSD's of 63% (vomitoxin) and 55% (T-2 toxin)

[na] not analysed

Balkan endemic nephropathy (BEN) in humans living in areas with high incidence of urinary tract tumors, a disease pattern known to co-occur with BEN (29–32). The urinary tract cancer incidence in BEN affected countries was 39 cases/100 000 population against 15 cases/100 000 population in countries, where BEN was non-endemic (33). The cancer incidence of kidney and urinary organs (excluding bladder) in the Ecuadorian population was 44 cases/100 000 for females and 48 cases/100 000 for males (20). The ingested dose of OA (based on arithmetic mean values) exceeds the estimated tolerable daily intake (0.2–4.2 ng/kg BW/d) based on carcinogenicity study in rats (22, 23, 34, 35). Considering only the renal damages and not taking account of any carcinogenic data, a provisional tolerable weekly intake of 110 ng/kg BW was established in 1991 from a Joint FAO/WHO Expert Committee on Food Additives (36). The mean levels of OA contamination in beans and corn in areas endemic for BEN are about three times higher compared to non endemic regions (37). Considering all the information given above, the frequencies and levels of contamination found in Ecuadorian corn and bean samples are suspected to be partly responsible for the high incidence of kidney and urinary tract tumors in the Ecuadorian population. Even when considering the small number of samples analysed by HPLC and thus being working with a poor estimate of the mean contamination level, it is seen that Ecuadorians ingest up to 20 fold the amount of OA ingested in most western European countries and that the contamination levels of foods are comparable with those from the Balkan region.

Trichothecenes

Based on the ELISA measurements, the daily intake of the trichothecenes (based on arithmetic mean values; values in paranthesis are based on medians) *T-2 toxin* and *DON* in Ecuador were estimated to be 76 (69) and 1330 (910) ng/kg BW/d, respectively (table 3). Existing tolerances for the trichothecenes are 100 ng/g for T-2 toxin in ex-USSR and 500–2000 ng/g for DON in Canada, the United States and the ex-USSR (4). DON rarely is found at contamination levels > 1 µg/g in human foodstuffs. Both trichothecene toxins are partially water soluble and are partially removed by boiling or wet milling (6). The acute toxicity of DON in animals is regarded to be 4 to 10 fold less than that of T-2 toxin, but the ingestion of contaminated feeds over prolonged periods may result in reduced body weight gain, feed refusal and diarrhea (38). In mice, total suppression of the immune response was observed at dosage of 2.5 mg T-2 toxin/kg BW (39). Only limited human toxicological data are available. Human liver enzymes deacetylate T-2 toxin *in vitro* (40). The different stages of alimentary toxic aleukia (ATA) disease, including hyperaemia of the oral mucosis, weakness, nausea and vomiting or, in severe cases, oesophagitis, gastritis, gastroenteritis, circulatory failure and convulsion, have been associated to the consumption of *Fusarium* contaminated foods (41). Outbreaks have been reported from China (42) and India (43) and were related to the consumption of corn and wheat contaminated with 0.3–93 mg DON/kg, but other trichothecenes were present at minor amounts. Animal data report increased pulmonary and hepatocellular adenoma and pancreatic tumors in mice and rats administered up to 3 µg T-2 toxin/kg BW (44). There is limited evidence in experimental animals for the carcinogenity of T-2 toxin and trichothecenes were judged not to be classifiable regarding to their cancerogenic potential to humans (6, 41).

It can be concluded, that the ingestion of the trichothecenes T-2 toxin and DON by the Ecuadorian population are not of significant immediate health concern.

Fumonisins

The estimated daily intake of fumonisins (based on arithmetic mean values; values in paranthesis are based on medians) by Ecuadorians reaches 447 (287) ng/kg BW/d (table 3). In Switzerland, the daily intake of fumonisins is estimated to be 30 ng/kg BW/d. In animals, varied toxicological effects are related to ingested doses from 0.125–5 mg/kg BW/d, including symptoms like brain necrosis, hepatotoxicity and pulmonary edema (10, 45, 46). Horses are at risk to develop equine leukoencephalo malicia (ELEM) when consuming feed contaminated with levels as low as 8 µg/g (47) and the Mycotoxin Commitee of the American Association of Veterinary Laboratory Diagnosticians recommends a limit of 5 µg/g fumonisin for feed to equidae (48). A cancerogenic activity of FB_1 has been found in rat liver (8, 46, 48, 49). However, the classification of FB_1 and FB_2 is «limited evidence» and «inadequate evidence» for animal cancerogenicity, respectively (49).

The ingestion of fumonisins might be relevant, but there is limited knowledge to judge about possible human health implications. However, Ecuadorians ingest

up to 20 fold the amount of OA ingested in most western European countries, the contamination levels of foods and also the cancer incidence of kidney and urinary tract organs in the Ecuadorian population are comparable with those from Balkan endemic nephropathy (BEN) regions.

Conclusions

The ingestion of aflatoxins, ochratoxin A and fumonisins in foods and feeds pose problems for human and animal health in Ecuador. The content of aflatoxin in rice is of importance, because rice is the most important staple food of the Ecuadorian population and none of the other analysed toxins were found to be present at high levels. Aflatoxins, ochratoxin A and vomitoxin were shown to be partially removed into the polish powder fraction. Hard endosperm corn proved to be a matrix of high risk for high aflatoxin (up to 6000 ng/g) and fumonisin (up to 8000 ng/g) contamination levels, specially when grown and stored in tropical hot, humid regions of the country. Furthermore, co-contamination with ochratoxin A occurs, which is supposed to be a cancerogen itself (7) and to be involved in Balkan endemic nephropathy (50). Aflatoxin and fumonisin contents clearly were related to high moisture contents. Mouldy corn had statistically significant higher aflatoxin contents compared to non mouldy corn, but this correlation does not hold true for ochratoxin A, the fumonisins and the trichothecenes T-2 toxin and DON.

Soft and medium hard endosperm corn as well as beans do not pose a first order problem concerning their aflatoxin or fumonisin contents, but high contaminations of ochratoxin A (up to 320 ng/g in corn and up to 80 ng/g in beans) and vomitoxin (up to 2 µg/g in corn and beans) occur. Especially susceptible for ochratoxin A contamination is the corn variety used to produce «mote», whereas the bean variety «Panamito» was susceptible for DON contamination. Post-harvesting programs concerning these matrices should be guided to avoid the disastrous losses by insect infestation and rodents during storage (51). Furthermore, it should be tested, if unadequate storage conditions are partially responsible for the high ochratoxin A contaminations in soft corn and beans. In none of the analysed matrices, trichothecene T-2 toxin was found at concentration levels which could represent any health hazard. The calculated aflatoxin, ochratoxin A and fumonisin ingestion by the Ecuadorian people should be analysed in parallel by clinical investigations such as AFB-albumin adducts in blood (52), ochratoxin A in the blood (23) and in the case of fumonisin exposure the ratio of sphinganine to sphingosine in blood serum (53). These suggested analysis of single blood samples would lead to a better estimation of the health hazards caused by the three mycotoxins mainly found in Ecuador.

Acknowledgements

We thank the Departement für auswärtige Angelegenheiten, Direktion für Entwicklung und Zusammenarbeit (DEZA) Bern, for the grant to M. Mühlemann; *Ch. Wahli, M. Koziol,*

S. von Rütte, J. Di Domenico and *L. Kistler* (all from LATINRECO S.A., Quito, Ecuador, Centro de desarrollo de alimentos de Nestlé) for laboratory, chemicals, reagents and support; *B. Zimmerli, O. Zoller, F. Sager* and *R. Dick* (all from the Federal Office of Public Health, Berne, Switzerland) for help in HPLC analysis of ochratoxin A, analysis of the fumonisin contents of 11 corn samples from Ecuador and the organisation of the interlaboratory study concerning thrichothecene analysis; *A. Pittet* from Nestlé SA in Switzerland for communicating unpublished results; *M. Teran* and *G. Pesantes* (both from ENAC) as well as *E. Basantes* and *J. Bravo* (both from MAG-FAO) for sampling permissions and facilities; *S. Espin de Rivera* from INIAP for reagents and information; *S. Balarezo* from CESA for introduction to practices of rice farmers; *F. Rohn* and *P. Sola* (both from CAAP) for nutritional information.

Summary

Between 1992 and 1994, different Ecuadorian foods and feeds (99, 89 and 79 samples of rice, corn and beans, respectively) were examined for mycotoxin contaminations (ochratoxin A, DON, T-2 toxin and fumonisin) by ELISA and HPLC. Regression analysis of ELISA vs HPLC results revealed a severe underestimation (50%) of naturally occurring ochratoxin A by ELISA in corn. The recoveries from artificially contaminated samples ranged from 73% (DON in beans) to 206% (T-2 toxin in beans) in ELISA and were up to 61% (ochratoxin A in corn) in HPLC analysis. Whereas unproblematic contamination levels of the trichothecenes and of the fumonisins were found, high contamination levels were found for ochratoxin A in corn and beans (both up to 320 ng/g). High ochratoxin A levels seem to be variety dependent in corn and the presence of moulds was not indicative of high toxin content. High fumonisin concentrations were detected in hard endosperm corn produced in the regions of the tropical cost and the east, and a correlation between moisture content and fumonisin contamination was found. Again, mould growth was not correlated with the presence of the toxin. Total intake of ochratoxin A, fumonisin, DON and T-2 toxin (based on arithmetic mean values; values in paranthesis are based on medians) by Ecuadorians were estimated to be 34 (3.4), 477 (287), 1330 (910) and 76 (69) ng/kg BW/day, respectively. The Ecuadorian practice of hygienic quality judgement by appearance and moisture content analysis cannot guarantee safety of the foodstuffs and therefore, basic regulatory and control actions should be taken.

Zusammenfassung

In den Jahren 1992 bis 1994 wurden verschiedene Grundnahrungs- und Futtermittel (99, 89 und 79 Reis-, Mais- bzw. Bohnenmuster) aus Ecuador auf den Gehalt an Mycotoxinen (Ochratoxin A, DON, T-2 Toxin und Fumonisin) mittels ELISA und HPLC untersucht. Die Regressionsanalyse ELISA versus HPLC-Analysewerte ergab eine drastische Unterschätzung um 50% von natürlich vorkommenden Ochratoxin-A-Gehalten in Mais mittels ELISA. Die Wiederfindungsraten von zugesetzten Trichothecenen lagen zwischen 73% (DON in Bohnen) und 206% (T-2 Toxin in Bohnen) beim ELISA und erreichten 61% (Ochratoxin A in Mais) bei der HPLC-Analytik. Währenddem nur unproblematische Kontaminationen von Trichothecenen und Fumonisinen gefunden wurden, traten hohe Kontaminationen von Ochratoxin A in Mais und Bohnen (jeweils bis 320 ng/g) auf. Die hohe Ochratoxin-A-Kon-

tamination von Mais erwies sich als sortenabhängig und unabhängig vom Vorhandensein von Schimmelpilzen. Die Hartmaisproben mit den höchsten Fumonisingehalten stammten aus der (tropischen) Küstenregion und der Ostregion Ecuadors, wobei eine positive Korrelation zwischen Feuchtigkeitsgehalt der Proben und Höhe der Fumonisingehalte festgestellt werden konnte. Hohe Fumonisinkontaminationen erwiesen sich als unabhängig vom Vorhandensein von Schimmelpilzen. Die mittlere Aufnahme (beruhend auf dem arithmetischen Mittelwert; der Median befindet sich in Klammern) von Ochratoxin A, Fumonisin, DON und T-2 Toxin wurde aufgrund dieser Daten und der Ernährungsgewohnheiten in Ecuador abgeschätzt: sie liegen im Bereich von (3,4)–34, (287)–477, (910)–1330 bzw. (69)–76 ng/kg Körpergewicht und Tag. Die Beurteilung der hygienischen Qualität mittels optischem Aspekt und Bestimmung des Feuchtigkeitsgehaltes kann die Sicherheit der Nahrungsmittel nicht garantieren. Massnahmen zur Verbesserung der Situation sind notwendig.

Résumé

Entre 1992 et 1994, la teneur en mycotoxines (ochratoxine A, déoxynivalénol (DON), T-2 toxine et fumonisine) de divers aliments de base pour humains et animaux (respectivement 99, 89 et 79 échantillons de riz, de maïs et d'haricots) en provenance de l'Equateur a été déterminée par ELISA et HPLC. L'analyse de régression comparative des résultats obtenus par ELISA et HPLC a révélé une sous-estimation importante, de 50%, par ELISA de la teneur en ochratoxine A naturellement présente dans la maïs. Les taux de récupération pour les trichothécènes ajoutés (contamination volontaire) se situent par ELISA entre 73% (DON dans les haricots); le même taux atteint 61% (ochratoxine A dans le maïs) par HPLC. Tandis que les contaminations par trichothécènes et fumonisines ne posent pas de problème, les contaminations par ochratoxine A dans le maïs et les haricots étaient très élevées (jusqu'à 320 ng/g). Il semblerait que les contaminations de maïs par l'ochratoxine A dépendent de la variété, mais non pas de la présence de moisissures. Les échantillons de grains durs de maïs avec les teneurs en fumonisines les plus élevées proviennent de la région de la côte tropicale et de la région orientale de l'Equateur; une corrélation positive a été constatée entre l'état hygroscopique et l'importance des teneurs en fumonisine de ces échantillons. Par contre, il n'y a pas de rapport entre des teneurs élevées en fumonisines et la présence de moisissures. L'absorption moyenne (elle se base sur la moyenne arithmétique; la valeur médiane se trouve entre parenthèses) d'ochratoxine A, de fumonisine, de DON et de T-2 toxine a été estimée en utilisant ces données et selon les habitudes nutritionnelles en Equateur: cette absorption journalière se situe dans le domaine de 34 (3,4), 477 (287), 1330 (910) et 76 (69) ng/kg de poids du corps. L'appréciation de la qualité hygiénique au moyen de l'aspect optique et par la détermination de la teneur en humidité ne peut pas garantir l'innocuité de ces aliments. Des mesures au niveau légal et dans le domaine assurance qualité sont donc nécessaires pour remédier à cette situation.

Literature

1. *Pohland, A.E.:* Mycotoxins in review. Food Additives and Contaminants **10**, 17–28 (1993).
2. *IARC:* Monograph on the evaluation of carcinogenic risk to humans. Suppl. **7**, 82–87. International Agency for Research on Cancer, Lyon 1987.

3. *Van Egmond, H.P.:* Mycotoxins: regulations, quality assurance and reference materials Food Additives and Contaminants **12**, 321–330 (1995).
4. *Van Egmond, H.P.:* Current situation on regulations for mycotoxins. Overview of tolerances and status of standard methods of sampling and analysis. Food Additives and Contaminants **6**, 139–188 (1989).
5. *Zoller, O., Sager, F.* und *Zimmerli, B.:* Vorkommen von Fumonisinen in Lebensmitteln. Mitt. Gebiete Lebensm. Hyg. **85**, 81–99 (1994).
6. *IPCS:* Selected mycotoxins: ochratoxins, trichothecenes, ergot in Environmental Health Criteria (ed. WHO) WHO, Geneva 1990.
7. *Huff, J.E.:* Carcinogenity of ochratoxin A in experimental animals. In: Mycotoxins, endemic nephropathy and urinary tract tumors (eds. Castegnaro, M., Plestina, R., Dirheimer, G., Chernozemsky, I.N. & Bartsch, H.) 229–244. International Agency for Cancer Research, Lyon 1991.
8. *Gelderblom, W.C.A., Kriek, N.P.J., Marasas, W.F.O.* and *Thiel, P.G.:* Toxicity and carcinogenicity of the *Fusarium moniliforme* metabolite, fumonisin B_1, in rats. Carcinogenesis **12**, 1242–1251 (1991).
9. *Marasas, W.F.O., Kriek, N.P.J., Fincham, J.E.* and *vanRensburg, S.J.:* Primary liver cancer and oesophageal basal cell hyperplasia in rats caused by *Fusarium moniliforme.* Int. J. Cancer **34**, 383–387 (1984).
10. *Marasas, W.F.O., Kellerman, T.S., Gelderblom, W.C.A., Coetzer, J.A.W.* and *Thiel, P.G.:* Leucoencephalomalacia in a horse induced by fumonisin B_1 isolated from *Fusarium moniliforme*. Onderstepoort J. Vet. Res. **55**, 197–203 (1988).
11. *Espin de Rivera, S.:* Informe in Taller Conjunto FAO/OPS sobre Prevención y Control de Micotoxinas en America Latina y el Caribe. INIAP, Quito 1991.
12. *Mühlemann, M., Lüthy, J.* and *Hübner, P.:* Mycotoxin contamination of food in Ecuador. A: Aflatoxins. Mitt. Gebiete Lebensm. Hyg. **88**, 474–496 (1997).
13. *Scott, P.M.:* Natural poisons in official methods of analysis (ed. Helrich, K.) 1184–1213. Assoc. Off. Anal. Chem., Inc., Arlington 1990.
14. *Steiner, W., Battaglia, R., Buxtorf, U.P., Cominoli, A., Guggisberg, H., Koch, H., Leuenberger, U., Lüthy, J.* et al.: Toxische Stoffe natürlichen Ursprungs in Schweiz. Lebensmittelbuch, 1. Teillieferung, Kapitel 54 (ed. BAG). Bern 1992.
15. *Baumann, U.* und *Zimmerli, B.:* Einfache Ochratoxin-A-Bestimmung in Lebensmitteln. Mitt. Gebiete Lebensm. Hygiene **79**, 151–158 (1988).
16. *Uchiyama, S., Saito, Y.* and *Uchiyama, M.:* Protein-binding of ochratoxin A and its extractability from proteinous food. J. Food Hyg. Soc. Japan **26**, 651–657 (1985).
17. *Zoller, O.:* Vergleichsanalyse Fusarientoxine (Trichothecene und Zearalenon). Unpublished Report. The Federal Office of Public Health (BAG), Berne 1996.
18. *Rivas, F., Caballero, D.* y *Llamuca, G.:* Determinación de agentes fungosos en differentes sistemas de almacenamiento en maíz (Zea mays, L) y frejol (Phaseolus vulgaris, L) en la zona de Pallatanga in Projecto de Postcosecha y Mercadeo Primario de Cereales y Papa, GCP/ECU/060/NET, documento de campo (ed. FIA-ESPOCH). FIA-ESPOCH, Riobamba 1992.
19. *MAG-PRSA:* Hoja de balance de alimentos, año 1991 in Proyecto Para la Reorientación del Sector Agropecuario (PRSA) (ed. MAG). MAG, Quito 1992.
20. *SOLCA:* Cancer en Quito Anuario 1990. Registro Nacional de Tumores, Quito 1992.
21. *Frank, H.K.:* Risk estimation for ochratoxin A in European countries. In: Mycotoxins, endemic nephropathy and urinary tract tumors (eds. Castegnaro, M., Plestina, R., Dir-

heimer, G., Chernozemsky, I.N. & Bartsch, H.) 321–325. International Agency for Research on Cancer, Lyon 1991.
22. *Kuiper-Goodman, T.* and *Scott, P.M.:* Risk assessment of the mycotoxin ochratoxin A. Biomed. Environm. Sciences **2**, 179–248 (1989).
23. *Zimmerli, B.* and *Dick, R.:* Determination of ochratoxin A at the ppt level in human blood, serum, milk and some foodstuffs by high-performance liquid chromatography with enhanced fluorescence detection and immunoaffinity column cleanup: methodology and Swiss data. J. Chromatogr. B **666**, 85–99 (1995).
24. *Breitholz, A., Olsen, M., Dahlbäck, A.* and *Hult, K.:* Plasma ochratoxin A levels in three Swedish populations surveyed using an ion-pair HPLC technique. Food Additives and Contaminants **8**, 183–192 (1991).
25. *Creppy, E.E., Betbeder, A.M., Gharbi, A., Counord, J., Castegnaro, M., Bartsch, H., Moncharmont, P., Fouillet, B.* et al.: Human ochratoxicosis in France. In: Mycotoxins, endemic nephropathy and urinary tract tumors (eds. Castegnaro, M., Plestina, R., Dirheimer, G., Chernozemsky, I.N. & Bartsch, H.) 145–151. International Agency for Research on Cancer, Lyon 1991.
26. *IARC:* Ochratoxin A in Monographs on the evaluation of the carcinogenic risks to humans. Some naturally occurring substances: food items and constituents, heterocyclic aromatic amines and mycotoxins (ed. IARC) 489–521. International Agency for research on Cancer, Lyon 1993.
27. *Fuchs, R., Radic, B., Ceovic, S., Sostaric, B.* and *Hult, K.:* Human exposure to ochratoxin A. In: Mycotoxins, endemic nephropathy and urinary tract tumors (eds. Castegnaro, M., Plestina, R., Dirheimer, G., Chernozemsky, I.N. & Bartsch, H.) 131–134. International Agency for Research on Cancer, Lyon 1991.
28. *Krogh, P.:* Mycotoxin porcine nephropathy: a possible model for Balkan endemic nephropathy. In: Endemic nephropathy (eds. Puchlev, A., Dinev, I.V., Milev, B. & Doichinov, D.) 266–270. Bulgarian Academy of Sciences, Sofia 1974.
29. *Chernozemsky, I.N., Stoyanov, I.S., Petkova-Bocharova, T.K., Nicolov, I.G., Draganov, I.V., Stoichev, I.I., Tanchev, Y., Naidenov, D.* and *Kalcheva, N.D.:* Geographic correlation between the occurrence of endemic nephropathy and urinary tract tumors in Vratza district, Bulgaria. International J. of Cancer **19**, 1–11 (1977).
30. *Sostaric, B.* and *Vukelic, M.:* Characteristics of urinary tract tumors in the area of Balkan endemic nephropathy in Croatia. In: Mycotoxins, endemic nephropathy and urinary tract tumors (eds. Castegnaro, M., Plestina, R., Dirheimer, G., Chernozemsky, I.N. & Bartsch, H.) 29–35. International Agency for Research on Cancer, Lyon 1991.
31. *Stojanov, I.S., Stojchev, I.I., Nicolov, I.G., Draganov, I.V., Petkova-Bocharova, T.K.* and *Chernozemsky, I.N.:* Cancer mortality in a region with endemic nephropathy. Neoplasma **24**, 625–632 (1977).
32. *Stojanov, I.S., Chernozemsky, I.N., Nicolov, I.G., Stoichev, I.I.* and *Petkova-Bocharova, T.K.:* Endemic association between endemic nephropathy and urinary system tumors in an endemic region. J. chronic Dis. **31**, 721–724 (1978).
33. *Radovanovic, Z.* and *Krajinovic, S.:* The Balkan endemic nephropathy and urinary tract tumors. Arch. Geschwulstforsch. **49**, 444–447 (1979).
34. *Boorman, G.:* Technical report on the toxicology and carcinogenesis studies of ochratoxin A in F334/n rats. National Toxicology Program, US Department of Health and Human Services, Tesearcg Triangle Park, North Carolina 1988.
35. *Kuiper-Goodman, T.:* Risk assessment of ochratoxin A residues in food. In: Mycotoxins, endemic nephropathy and urinary tract tumors (eds. Castegnaro, M., Plestina, R., Dir-

heimer, G., Chernozemsky, I.N. & Bartsch, H.) 307–320. International Agency for Research on Cancer, Lyon 1991.
36. *WHO:* Evaluation of certain food additives and contaminants. 37th Report of the Joint FAO/WHO Expert Comitee on Food Additives. In: WHO Technical Report Series 806 (ed. WHO) 28–31. WHO, Geneva 1991.
37. *Petkova-Bocharova, T.* and *Castegnaro, M.:* Ochratoxin A in relation to Balkan endemic nephropathy and urinary tract tumors in Bulgaria. In: Mycotoxins, endemic nephropathy and urinary tract tumors (eds. Castegnaro, M., Plestina, R., Dirheimer, G., Chernozemsky, I.N. & Bartsch, H.) 135–137. International Agency for Research on Cancer, Lyon 1991.
38. *Cvirn, G., Murkovic, M., Pfannhauser, W., Lew, H.* und *Lindner, W.:* Zearalenon und Deoxynivalenol in österreichischem Getreide. Mitt. Gebiete Lebensm. Hyg. **85**, 728–736 (1994).
39. *Rosenstein, Y., Lafarge-Frayssinet, C., Lespinats, G., Louisillier, F., Lafont, P.* and *Frayssinet, C.:* Immunosuppressive activity of *Fusarium* toxins. Effects on antibody synthesis and skin grafts of crude extracts of T-2 toxin and diacetoxyscirpenol. Immunology **36**, 111–118 (1979).
40. *Ellison, R.A.* and *Kotsonis, F.N.:* In vitro metabolism of T-2 toxin. Appl. Microbiol. **27**, 423–424 (1974).
41. *IARC:* Toxins derived from *Fusarium* sporotrichioides: T-2 toxin. In: Monographs on the evaluation of carcinogenic risk to humans. Some naturally occurring substances: food items and constituents, heterocyclic aromatic amines and mycotoxins (ed. IARC) 467–487. International Agency for Research on Cancer, Lyon 1993.
42. *Luo, X.:* Food poisoning associated with *Fusarium* toxins. In: Proceedings of the 7th International Symposium on Mycotoxins and Phycotoxin. Tokyo, 16–19 August, 1988.
43. *Bhat, R.V., Beedu, S.R., Ramakrishna, Y.* and *Munshi, K.L.:* Outbreak of trichothecene mycotoxicosis associated with consumption of mould-damaged wheat products in Kashmir valley, India. Lancet **7**, 35–37 (1989).
44. *Schoental, R., Joffe, A.Z.* and *Yagen, B.:* Cardiovascular lesions and various tumors found in rats given T-2 toxin, a trichothecene metabolite of *Fusarium*. Cancer Res. **39**, 2179–2189 (1979).
45. *Harrison, L.R., Colvin, B.M., Green, J.T., Newman, L.E.* and *Cole, J.R.:* Pulmonary edema and hydrothorax in swine produced by fumonisin B_1, a metabolite of *Fusarium moniliforme.* J. Vet. Diagn. Invest. **2**, 217–221 (1990).
46. *Haschek, W.M., Motelin, G., Ness, D.K., Harlin, K.S., Hall, W.F., Vesonder, R.F., Peterson, R.E.* and *Beasley, V.R.:* Characterisation of fumonisin toxicity in orally and intravenously dosed swine. Mycopathologia **117**, 83–96 (1992).
47. *Wilson, T.L., Ross, P.F., Owens, D.L., Rice, L.G.* and *Green, S.A.:* Experimental reproduction of ELEM – a study to determine the minimum toxic dose in ponies. Mycopathologia **117**, 115–120 (1992).
48. *Riley, T.L., Norred, W.P.* and *Bacon, C.W.:* Fungal toxins in foods: recent concerns. Annu. Rev. Nutr. **13**, 167–189 (1993).
49. *IARC:* Toxins derived from *Fusarium moniliforme*: Fumonisins B_1 and B_2 and fusarin C. In: Monographs on the evaluation of carcinogenic risk to humans. Some naturally occurring substances: food items and constituents, heterocyclic aromatic amines and mycotoxins (ed. IARC) 445–466. International Agency for Research on Cancer, Lyon 1993.
50. *Bach, P.H., Gregg, N.J.* and *Delacruz, L.:* Relevance of a rat model of papillary necrosis and upper urothelial carcinoma in understanding the role of ochratoxin A in Balkan

Endemic Nephropathy and its associated carcinoma. Food and Chemical Toxicol. **30**, 205–211 (1992).
51. *FAO:* Memorias del seminario-taller 'Problemas y alternativas del sector agroalimentario' in Projecto de Postcosecha y Mercadeo Primario de Cereales y Papa, GCP/ECU/060/NET; Documento de Campo (ed. MAG-FAO). FAO, Quito 1992.
52. *Groopman, J.D.:* Molecular dosimetry methods of assessing human aflatoxin exposures. In: The toxicology of aflatoxins: Human health, veterinary and agricultural significance (eds. D. L. Eaton & Groopman, J.D.) 259–279. Academic Press, Inc., San Diego 1994.
53. *Wang, E., Ross, P.F., Wilson, T.M., Riley, R.T.* and *Merril, A.H.:* Increases in serum sphingosine and sphinganine and decrease in complex sphingolipids in ponies given feed containing fumonisins, mycotoxins produced by *Fusarium moniliforme.* J. Nutr. **122**, 1706–1716 (1992).

Dr. Marc Mühlemann[1]
PD Dr. Jürg Lüthy
Dr. Philipp Hübner[2]
Labor für Lebensmittelchemie
Departement für Chemie und Biochemie
Universität Bern
Freiestrasse 3
CH-3012 Bern

[1] present address: Utreras 709 y S. Alegre, Quito-Ecuador
[2] corresponding author