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Levels of mycotoxins and sample cytotoxicity of selected organic and conventional grain-based products purchased from Finnish and Italian markets

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The contamination levels of 16 different *Fusarium*- and *Aspergillus*-mycotoxins were chemically determined from randomly selected organic and conventional grain-based products purchased from Finnish and Italian markets. The cytotoxicity of the samples was analyzed with an *in vitro* test using feline fetal lung cells. Overall, the concentrations of the mycotoxins studied were low in all of the samples. Enniatins B and B1 as well as deoxynivalenol were the most predominant mycotoxins in the samples, being present in 97%, 97%, and 90% of the samples, respectively. The geographical origin or the agricultural practice had no influence on the mycotoxin concentrations of the samples. The babyfoods included in the samples had significantly lower concentrations of mycotoxins than the other products with a mean total mycotoxin content of 47 μg/kg compared with 99 μg/kg for the other kinds of food. All the samples evoked toxicity in the *in vitro* test, but no correlation between cytotoxicity and the mycotoxin concentrations was observed.

Keywords: Cytotoxicity / Grain-based products / Mycotoxins / Organic agriculture

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1 Introduction

The production of organic foods in Europe increased more than fivefold between 1993 and 2000 [1]. The great majority of the organic foods in European markets are grain-based products, and these have also the highest market shares (2.2% of the total) of all organic products. In Italy and Finland, the consumption of grain-based organic products is higher than the European mean value [1]. Grain-based products are one of the main sources of mycotoxins in both the human and animal diets. The mean European consumption of all cereals and cereals-based products (excluding beer) is 133.1 kg *per capita* [2]. The possible health risks caused by food contaminants like heavy metals [3 – 5], nitrate/nitrite [5], and mycotoxins [5 – 8] in organic products have been examined in a number of studies. The results obtained are quite inconsistent: some studies do not show any differences between organic or conventional products, while some have reported increased risk from consumption of organic foods and others state that conventional foods pose a greater risk. In fact, most studies have concluded that more investigations are needed before we can assess the safety of these products. Despite the possible risks involved in organic food production, consumers perceive them as pro-environmental and safe, although according to Kouba [9] epidemiological studies are needed to demonstrate the possible positive or negative health effects of organic foods.

Mycotoxins are secondary metabolites produced under favorable conditions by filamentous fungi, *e.g.*, *Fusarium* spp., *Aspergillus* spp., and *Penicillium* spp. Mycotoxins may pose a health risk, *e.g.*, some of them are recognized mutagens and carcinogens. Organic agricultural practices do not allow the use of chemical products such as fungicides and growth regulators. This may enable the colonization of fungi with the consequent increase in mycotoxin production and their accumulation in the crops. The prohibited use of fungicides promotes the exposure of cereal grains to infections. In addition, without growth regulators, the fields are more susceptible to lodging and decay. All these factors

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Abbreviations: **AB_1**, aflatoxin B_1; **3AcDON**, 3-acetyldeoxynivalenol; **BEA**, beauvericin; **CR**, cytotoxic response; **DAS**, diacetoxyscirpenol; **DON**, deoxynivalenol; **ENNs**, enniatins (A, A1, B, B1); **FL**, feline fetal lung cells; **FUS**, fusaproliferin; **FX**, fusarenon X; **HT-2**, HT-2-toxin; **MON**, moniliformin; **NIV**, nivalenol; **T-2**, T-2-toxin; **ZEN**, zearalenone

may expose cereals to fungal infections by especially *Fusarium* fungi, which are the main mycotoxin producers in field conditions. Furthermore, the direct sowing method commonly engaged in organic agriculture predisposes the crops to early contamination by toxigenic fungi in the fields. On the other hand, organically cultivated grains may have lower concentrations of mycotoxins, because the crop rotation routinely used prevents the transmission of plant diseases.

Until very recently, about 95% of all chemical toxicity studies were performed using single chemicals. However, studies involving chemical mixtures are of interest due to concerns of public health [10]. The toxicology of chemical mixtures like cigarette smoke, dioxin-like products, and mycotoxins is a complex question due to the possible additive, synergistic, or antagonistic effects of the individual compounds present in the mixture. Several approaches have been presented for the evaluation of the toxicological effects of mixtures [11]. These include different study designs, statistical models, and data analysis methods. If the detailed composition of the mixture as well as the toxicology of single compounds is known, a so-called bottom-up approach can be used. One application of this technique in risk assessment is the use of toxic equivalency factors (TEFs), with dioxin congeners as an example [12]. However, the bottom-up model is not applicable to complex mixtures consisting of several unknown components with a possible wide range of toxicological effects and in these cases, a top-down approach is more sound in the toxicological evaluation of the mixture as such. However, the broad diversity of the chemical and biological nature of mycotoxins makes the toxicological evaluation of these mixtures problematic but a top-down approach may be applicable to evaluate the real health risk related to food and its mycotoxin contents.

In vitro toxicity tests are an economical and fast way to screen for the toxicological effects of chemical mixtures because they eliminate the need to use laboratory animals and enable the evaluation of many samples under similar test conditions. In cytotoxicity tests, the viability of the cell line is tested in the presence of possible toxicants. The endpoint of the test can be, in general, based on visual inspection or on staining of cells for viability or reduced cell multiplication. With more specific end-points one can attempt to assess the variable properties of the toxic compound, *e.g.*, inhibition of DNA or protein synthesis, modifying the permeability of the cell membranes or decreasing of the cellular enzymatic activity, and in this way lower detection limits may be achieved. The cytotoxicity of selected mycotoxins has been studied with a number of different cell lines and end-points as reviewed by Gutleb *et al.* [13].

Though we have selected a top-down model to better understand the toxic effects of mycotoxin mixtures, accurate chemical analyses of compounds of interest clearly complement *in vitro* toxicology tests. Recent developments in analytical methods and techniques have enabled the detection of very low concentrations of mycotoxins. In this study, we determined mycotoxin contamination levels with sensitive mass spectrometric or fluorometric techniques (for details see Section 2). The cytotoxicity was also assessed for randomly selected grain-based products available on the Italian and Finnish markets.

2 Materials and methods

2.1 Samples

Randomly selected grain-based products were purchased from local markets in May 2002. 18 Finnish samples (7 organic) and 12 Italian samples (8 organic) were ground with a laboratory mill, if necessary, and stored at +4°C until analyzed for mycotoxin contamination and cytotoxic activity. The samples are presented in detail in Table 1.

2.2 Analysis of mycotoxins

Trichothecenes (deoxynivalenol (DON), fusarenon (FX), 3-acetyldeoxynivalenol (3-AcDON), diacetoxyscirpenol (DAS), nivalenol (NIV), HT-2 toxin (HT-2), T-2 toxin (T-2)) and fusaproliferin (FUS) were analyzed using GC-MS [14]. The LOQs were 10 μg/kg for DON, FX, 3-AcDON, and DAS, 20 μg/kg for HT-2 and T-2, 30 μg/kg for NIV, and 100 μg/kg for FUS. Beauvericin (BEA) and enniatins (ENNs) were analyzed with LC-MS/MS [30]. The LOQs for BEA, ENN A, ENN A1, ENN B, and ENN B1 were 10, 0.6, 4, 3.8, and 10.8 μg/kg, respectively. Sample numbers 1 – 5 had a higher water content and for this reason they were extracted with 100% ACN to increase the solvation power of the extraction solvent for trichothecenes and BEA and ENNs. Zearalenone (ZEN) was analyzed with HPLC using fluorescence detection after immunoaffinity column purification [31]. The LOQ for ZEN was 6 μg/kg, except for samples 19 and 23 (100 μg/kg) both of which were claimed to contain roasted barley according to the manufacturer. Moniliformin (MON) was analyzed with a triple quadrupole LC-MS [18]. The LOQ for MON was 20 μg/kg, except for samples 19 and 23 (100 μg/kg). Aflatoxin B_1 (AB_1) was analyzed with HPLC using fluorescence detection after immunoaffinity column purification [32]. The LOQ for AB_1 was 5 μg/kg.

2.3 Quality assurance for the mycotoxin analyses

All samples were analyzed using in-house validated methods for grains. As the texture of some samples was different from that of flour, recovery studies at one concentration level (Table 2) for each mycotoxin determined were performed during routine analyses by spiking some of these samples with standard solutions and analyzed according to

Table 1. Origin, cultivation practice, and the main ingredients of the samples

Sample No.	Sample	Origin (F/I)	Agricultural practice (O/C)	Main ingredients
1	Ready-to-eat baby-food	F	O	Oats, rice, carrot, apple
2	Ready-to-eat baby-food	F	O	Oats, wheat, rye, barley, blueberry, raspberry
3	Ready-to-eat baby-food	F	C	Wholemeal wheat, boysenberry
4	Ready-to-eat baby-food	F	C	Oats, wheat, rye, barley, pineapple, apple
5	Ready-to-eat baby-food	F	C	Oats, wheat, rye, barley
6	Infant cereal	F	C	Oats, maize, rice
7	Infant cereal	F	C	Oats, rice, pear
8	Infant cereal	F	C	Wholemeal wheat, pear, rice, oats, banana
9	Flour	F	O	Wheat
10	Flour	F	O	Rye
11	Flour	F	O	Barley
12	Oatmeal	F	O	Oats
13	Müsli	F	O	Wheat, oats, rye, honey
14	Flour	F	C	Wheat
15	Flour	F	C	Rye
16	Flour	F	C	Barley
17	Oatmeal	F	C	Oats
18	Müsli	F	C	Oats, wheat, barley, maize
19	Instant-drink powder	I	C	Toasted barley
20	Flour	I	C	Wheat
21	Infant cereal	I	C	Rice, barley, oats
22	Infant cereal	I	C	Rice, maize, orange, banana
23	Instant-drink powder	I	O	Toasted barley
24	Flour	I	O	Wheat
25	Flour	I	O	Spelt
26	Flour	I	O	Oats
27	Müsli	I	O	Oats, sultana, banana
28	Infant cereal	I	O	Oats, barley, rice, maize
29	Müsli	I	O	Oats, sultana, orange, banana
30	Müsli for babies	I	O	Barley, rice, maize, oats, apple, nuts

F = Finnish, I = Italian, O = organic, C = conventional

the original methods to ensure their applicability for the determination of selected mycotoxins in the samples.

2.4 Cytotoxicity test

Samples (25 g) were extracted with 100 mL 84% ACN in water (sample numbers 1 – 5: 100% ACN) and filtered. The filtered extract (10 mL corresponding to 2.5 g of sample) was evaporated to dryness under nitrogen flow and subsequently dissolved in 250 μL or 500 μL of 10% methanol in PBS, pH 7.2. A continuous feline fetal lung (FL) cell line (National Veterinary and Food Research Institute, Helsinki, Finland) was maintained in Eagle's Minimum Essential Medium (MEM) supplemented with 10% fetal calf serum, penicillin (100 IU/mL), streptomycin (100 μg/mL) and L-glutamine (2 mM). For the test, a confluent FL cell culture was trypsinized, and the cells dispersed in MEM (2.5×10^4 cells/mL). MEM (50 μL) was added to all wells and 50 μL sample extract (10% methanol PBS in control) were added to the first row of flat-bottomed, 96-well microplate. Each sample was tested in duplicate. Samples and controls were diluted from 1 : 2 to 1 : 256 using a serial micropipette and FL cell suspension (50 μL) was added to all wells. The plates were incubated at +37°C in humidified 5% CO_2 atmosphere for 3 days. The plates were assessed visually for cytotoxicity (cell death or diminished growth of cells). For the samples analyzed, the cytotoxic response (CR) was calculated. It was determined as an inverse number of the sample amount (in grams) that caused toxic effects to the cell line taking into account the possible dilutions. The higher the cytotoxic response, the smaller amount of crude extract was needed to induce necrosis, apoptosis or to reduce the viability of the cells.

2.5 Statistical analyses

The results from mycotoxin analyses and the cytotoxicity test were subjected to statistical analysis (Spearman rank correlation and one-way analysis of variance (ANOVA)) using Statistix for Windows, Version 2.0 (Analytical Software, Tallahassee, FL, USA). The p-values < 0.05 were considered statistically significant.

Table 2. Recoveries (%) of the analytes in different samples

Sample	DON	FX	3AcDON	DAS	NIV	HT2	T2	FUS	ZEN	MON	BEA	ENN A	ENN A1	ENN B	ENN B1	AB_1
1	55	155	138	182	93	183	158	67	96	45	31	72	74	61	106	31
4	56	203	130	153	110	172	142	61	84	50	71	64	68	95	116	25
7	80	115	100	118	83	108	108	35	113	63	77	98	98	103	155	
12	112	90	110	93	72	82	88	50	100	94	76	90	94	82	153	40
13	85	115	102	108	73	97	102	47	111	94	68	70	89	124	170	
17	107	120	118	118	75	105	103	46	98	98	80	89	97	64	149	
18	107	142	125	135	102	160	112	57	101	77	82	102	99	95	184	35
19	118	118	247	167	132	158	197	87	114[a)]	15	59	75	72	30	90	26
22	100	77	105	110	50	92	92	45	80	49	76	83	93	92	162	16
23	95	105	105	100	85	105	123	70	127[a)]	20	62	96	78	6	180	
27	88	138	117	117	72	113	95	82	92	80	59	75	77	48	119	
29	115	135	110	118	105	120	120	47	117	68	52	72	72	27	102	
30	85	97	103	103	78	87	102	51	120	83	51	60	66	40	104	
Sp. level (μg/kg)	60	60	60	60	60	60	60	600	25	100	100	6	40	38	108	50

a) Spiking level 100 μg/kg

3 Results and discussion

Differences between the recoveries were noted for most of the analytes in the samples with varying textures if compared to the in-house validation tests with grain as the matrix (data not shown), although no strict correlation between the type of mycotoxin being analyzed and the corresponding recovery was obtained. For example, the recoveries of DON, MON, and BEA from sample numbers 1 and 4 with a high water content were rather low, while for some trichothecenes the recovery was comparable and higher than for other samples. However, the recoveries of the analytes from the samples were considered to be analytically adequate. The recoveries of the analytes in different samples are summarized in Table 2. A relatively high recovery of trichothecenes (>100 % for most of the analytes) in many samples was detected. This is attributable to the differences in the sample preparation of matrix standards and samples as described by Jestoi *et al.* [14]. The recovery of some toxins from samples 19 and 23 (instant-drink powder) was rather poor, which led to some higher LOQs for these two samples. The recoveries of the other mycotoxins were, however, adequate in all samples and proved that the methods were applicable to the analysis of these mycotoxins in the samples. The recoveries obtained for some mycotoxins, for example, MON, ZEN, and AB_1, demonstrate that each matrix has its own distinct behavior and this means that specific extraction analysis protocols have to be adapted and optimized for each combination of mycotoxin and food.

The most common mycotoxins in the samples analyzed were ENN B (incidence 97% of the samples analyzed), ENN B1 (97%) and DON (90%). BEA, ENN A, ENN A1, MON, 3AcDON, NIV, and HT-2 were detected in 57%, 30%, 70%, 17%, 7%, 7%, and 13% of the samples, respectively. FX, DAS, T-2, FUS, ZEN, and AB_1 were not detected in any of the samples. The concentrations of the different mycotoxins detected in the samples are presented in Table 3.

The highest concentrations of ENNs were detected in Finnish rye flour (sample 15) produced by using traditional agricultural practices. Recently, we reported that ENN B and ENN B1 were present in all Finnish raw cereal samples thus far analyzed [15]. Also in this study we observed that all Finnish samples contained these toxins at low concentrations. ENN B and ENN B1 were found also in Italian samples as common contaminants: only one sample (sample 22) was not contaminated. DON was a common low concentration contaminant of the grain-based products in both countries.

BEA was detected at a concentration which was lower than the LOQ (10 μg/kg). Earlier studies have demonstrated that BEA is commonly found as a natural contaminant in Finland and Italy [15 – 17]. In this study, the concentrations are much lower than those previously reported from Italy. It is worth mentioning that the samples in our study were on sale in the markets and were not visibly contaminated with moulds, as was the case for the Italian investigation [17]. The Finnish raw cereal samples contained also very low concentrations of BEA [15]. However, higher concentrations have been reported in Finnish grains [16].

MON has been reported as a common contaminant of Finnish grains [15, 18]. However, in our study, the level of moniliformin was low and in most of the samples MON could not be detected. MON has been found to be stable and not to be destroyed during food processing [19], but it is possible that grinding of the samples may eliminate most of the MON contamination. The location of the infection of the fungi in grain, the consequent production of MON and its behavior during the milling process will require further investigations.

Even though *Fusarium* contamination in food commodities is common all around the world, the concentrations of *Fusarium* toxins were rather low in this study. This observation can be explained by the fact that there may have been

Table 3. Mycotoxin concentrations detected in samples (μg/kg)

Sample	Origin/practice[a)]	BEA	ENN A	ENN A1	ENN B	ENN B1	MON	DON	3AcDON	NIV	HT-2
1	F/O	–	–	–	< 3.8	< 10.8	–	29	–	–	–
2	F/O	–	< 0.6	< 4	26	< 10.8	–	46	–	–	–
3	F/C	–	–	–	10	< 10.8	–	29	–	–	–
4	F/C	< 10	–	–	< 3.8	< 10.8	–	26	–	–	–
5	F/C	–	–	< 4	9	< 10.8	–	–	–	–	–
6	F/C	< 10	–	< 4	< 3.8	< 10.8	–	< 10	–	–	–
7	F/C	–	–	< 4	< 3.8	< 10.8	–	< 10	–	–	< 20
8	F/C	–	–	< 4	< 3.8	< 10.8	–	< 10	–	–	–
9	F/O	< 10	1	< 4	37	20	25	14	–	–	–
10	F/O	< 10	5	15	99	50	21	13	–	–	< 20
11	F/O	–	< 0.6	< 4	19	< 10.8	–	< 10	–	–	–
12	F/O	< 10	–	< 4	< 3.8	< 10.8	–	63	16	–	–
13	F/O	< 10	1	< 4	11	< 10.8	–	39	–	–	–
14	F/C	–	< 0.6	< 4	63	26	42	42	–	–	–
15	F/C	< 10	10	20	170	71	< 20	23	–	–	–
16	F/C	–	2	< 4	26	14	–	< 10	–	–	–
17	F/C	< 10	–	< 4	< 3.8	< 10.8	–	22	–	–	–
18	F/C	< 10	< 0.6	4	11	< 10.8	–	15	–	–	20
19	I/C	–	–	–	< 3.8	< 10.8	–	–	–	79	23
20	I/C	< 10	–	< 4	< 3.8	< 10.8	–	27	–	–	–
21	I/C	–	–	–	< 3.8	< 10.8	–	21	–	–	–
22	I/C	–	–	–	–	–	–	< 10	–	–	–
23	I/O	–	–	–	< 3.8	< 10.8	–	–	–	–	–
24	I/O	< 10	–	< 4	< 3.8	< 10.8	–	< 10	–	–	–
25	I/O	< 10	–	–	< 3.8	< 10.8	–	20	–	–	–
26	I/O	< 10	–	–	< 3.8	< 10.8	–	107	–	–	–
27	I/O	< 10	–	< 4	< 3.8	< 10.8	< 20	37	–	–	–
28	I/O	< 10	–	< 4	< 3.8	< 10.8	–	13	< 10	< 30	–
29	I/O	< 10	–	< 4	< 3.8	< 10.8	–	< 10	–	–	–
30	I/O	< 10	–	< 4	< 3.8	< 10.8	–	41	–	–	–

FX, DAS, T-2, FUS, ZEN. and AB_1 were not detected in any of the samples; –, not detected; <, concentration below the LOQ
a) F, Finnish; I, Italian; O, organic; C, conventional

unfavorable growth or toxin production conditions for the moulds during the year that the samples were collected. The level of mycotoxins in contaminated raw materials has been shown to decrease or redistribute slightly during food processing such as wet and dry milling or grain cleaning [20–22]. This may partly explain the low concentrations observed in the processed samples.

In general, the mean concentration of total mycotoxins in the positive samples was higher in the Finnish samples compared to Italian samples (Fig. 1 a), but the geographical origin of the samples had no statistical significance on the mycotoxin concentrations. The incidence of ENNs and MON were higher in Finnish products, but these differences were also not statistically significant. Furthermore, the agricultural practice did not have any statistically significant effect on the total mycotoxin concentrations, although the mean concentration of total mycotoxins in the positive samples was slightly higher for organic products (Fig. 1 b). If all the samples together were assessed, then a statistically significant correlation was found between the concentrations of MON and ENNs, which is in line with the results of Jestoi *et al.* [15], who described a correlation between MON and ENNs in Finnish grain samples. Thus, MON and ENNs may well be produced by the same fungi. Alternatively, they may be produced by different fungi under similar environmental conditions.

Infants may be an at risk-group concerning mycotoxins because their diet is comprised predominantly of grain-based products. On this basis, we studied also baby-food samples (4 organic, 8 conventional). The mean mycotoxin concentrations of the positive samples was significantly lower in the baby-foods than in the products for general use (Fig. 1 c). With respect to the individual toxins, no statistical significance could be seen. DON, ENN B, and ENN B1 were detected in 92% of the baby-food samples. The most toxic mycotoxins determined, T-2, AB_1, and MON, were not detected in any of the samples and HT-2 and NIV each in one sample only (samples 7 and 28, respectively). Lombaert *et al.* [23] studied the mycotoxin concentrations in infant cereal food in Canadian markets and detected DON in 63% and ZEN in 33% of the samples analyzed. These findings along with ours indicate that diets of infants throughout the world do contain low levels of different mycotoxins. Despite this fact, the possible risk posed by the mycotoxins to babies is difficult to assess, because the concentration levels detected do not induce acute symptoms. The effects of long-term exposure of single mycotoxins as well as mycotoxin mixtures still needs to be studied. In Finland, the relative consumption of baby-foods is the highest in the world [24], so proper risk management practices are needed there to minimize the intake of mycotoxins by infants. In conclusion, baby-foods appear to be quite safe in terms of their mycotoxin content, but these results should not replace the need for constant surveillance, and more studies are needed to evaluate the effects from chronic exposure to mycotoxins.

The legislation concerning mycotoxins in food items is currently under intense development. The European Commis-

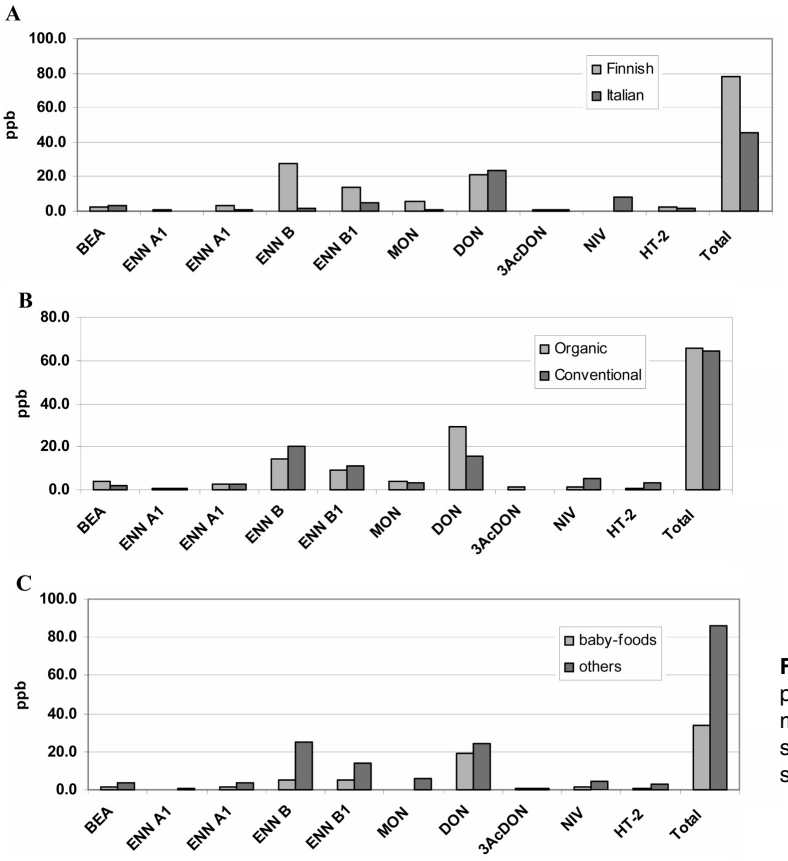

Figure 1. Effect (A) of the origin, (B) agricultural practice, and (C) the use of the samples on the mean mycotoxin concentrations of the positive samples. The concentrations below LOQ were considered as 0.5 × LOQ.

Table 4. Maximum levels set[a] or proposed[b] by the European Commission for grain-based products for human consumption

Mycotoxin	Purpose of use	Maximum level (μg/kg)
DON	Cereals for direct human consumption or use as food ingredient	500[b]
	Wholemeal wheat flour, bran, and pasta	750[b]
	Cereal based food for infants and young children	100[b]
T-2 + HT-2	Cereals (except oats)	100[b]
	Cereal products derived from oats	200[b]
	Cereal based food for infants and young children	50[b]
ZEN	Cereals for direct human consumption or use as food ingredient	50[b]
	Cereal-based food for infants and young children	20[b]
Fumonisin B_1 + fumonisin B_2	Maize for direct human consumption or use as food ingredient	500[b]
	Maize-based breakfast cereals, and maize-based snacks, and gluten-free cereal-based foods	200[b]
	Maize-based food for infants and young children	100[b]
AB_1	Cereals for direct human consumption or use as food ingredient	2[a]
	Cereal-based food for infants and young children	0.05[b]
Ochratoxin A	Cereals for direct human consumption or use as food ingredient	3[a]
	Cereal-based food for infants and young children	0.20[b]

sion has set a maximum level for AB_1 and ochratoxin A in selected foodstuffs. The maximum levels of DON, HT-2+T-2, ZEN, and fumonisins are under consideration at the moment. The maximum levels of the other toxins analyzed in our study will not be set in the near future, due to the lack of the data on toxicity, occurrence and contamination levels. There are national maximum levels in use, *e.g.*, for DON and fumonisins, and foodstuffs exceeding the national

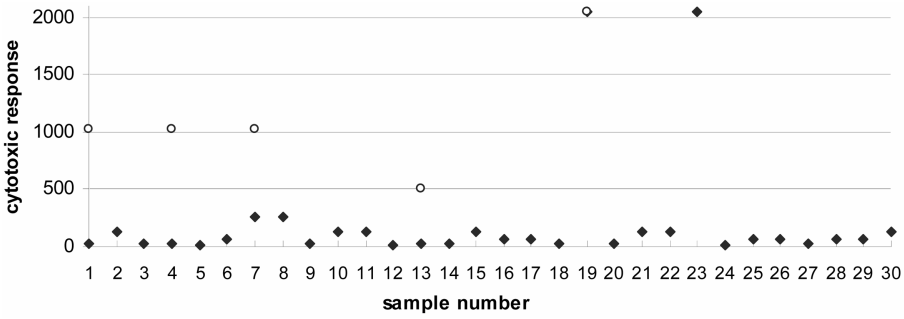

Figure 2. Cytotoxic response of the sample extracts on FL cells. The positive controls (samples spiked with trichothecene and fusaproliferin mixture) are marked as transparent circles. For sample description, please refer to Table 1.

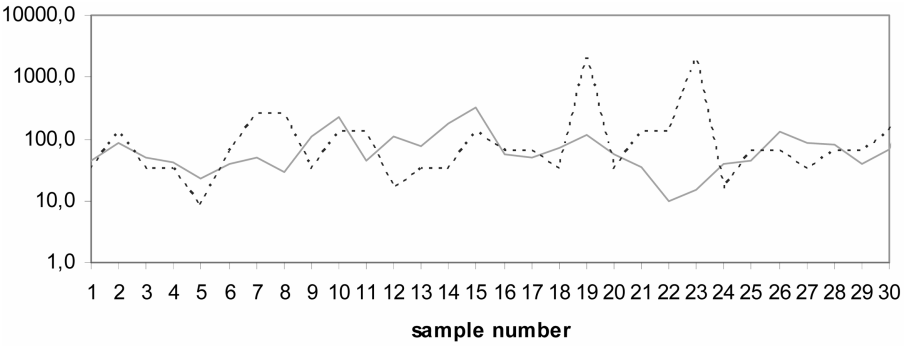

Figure 3. Total mycotoxin concentrations in μg/kg (continuous line) and the cytotoxic response (dotted line) of the samples. Note the logarithmic scale of the *y*-axis. The concentrations of the mycotoxins below the LOQ were considered as 0.5 × LOQ. For sample description, please refer to Table 1.

threshold values can be prohibited by the local food safety authorities. The concentrations of mycotoxins in our study were below any of the varying threshold values set or proposed by the European Commission for grain-based products (Table 4). The threshold value depends on the use of grains, *e.g.*, the lowest levels are proposed for cereal based foods for infants and young children.

For the cytotoxicity tests several primary and continuously growing mammalian cell lines were tested for their sensitivity to mycotoxins (data not shown). No resistant cell lines were found, but there was a slight variation in the sensitivity. The feline FL cells, continuously growing small fibroplasts having a high planting efficiency, were selected for the cytotoxicity assays. In general, FL-cells needed 2- to 10-fold lower concentrations of mycotoxins than the other cell lines tested to indicate toxic effect. FL cells also formed a uniform monolayer on which cytolytic changes could be easily observed.

CR was determined as an inverse number of the sample amount (in grams) that caused toxic effects to the cell line taking into account the possible dilutions. The higher the cytotoxic response, the smaller amount of crude extract was needed to induce necrosis, apoptosis or to reduce the viability of the cells. All sample extracts evoked toxicity in the feline FL cells (CR range 8–2048, mean 209) but the CR was clearly higher for the samples spiked with trichothecenes. The results of the cytotoxicity tests with FL-cells are presented in Fig. 2.

No statistically significant difference was observed between the cytotoxic response and geographical origin, the intended use or the agricultural practice, *i.e.*, organic or conventional farming. The cytotoxicities of the samples spiked with trichothecenes (60 μg/kg) and FUS (600 μg/kg) were elevated compared with the unspiked samples (Fig. 2). This demonstrates the sensitivity of the method and confirms that certain concentrations of mycotoxins can induce cytotoxicity in FL-cells. The highest cytotoxic response (CR = 2048) was observed for the two instant-drink powders, which had been processed by roasting barley grains (samples 19 and 23). Though it is an important factor in the production of aroma compounds, the high temperature used in the roasting process may promote the formation of new unwanted toxic by-products in reactions between sugar, lipid and/or protein fractions of the raw material [25]. Elevated toxicity could also be observed in some samples, especially in baby-foods, that contained berries or fruits (samples 2, 7, 8, 22, 30). The cytotoxicity of these samples may be due to the organic acids present in the ingredients.

The statistical analyses showed that the CR of the sample extracts could not be explained by the mycotoxins present in the samples (Fig. 3). However, the correlation between cytotoxic response and HT-2 was statistically significant. The statistical power was related to sample number 19, which was exceptionally toxic and contained a small amount of HT-2. However, the toxicity of this sample may be attributable to factors (*e.g.*, toxic by-products of Maillard reaction formed during roasting) other than mycotoxins.

There are a few studies on the toxicity of mycotoxin mixtures [26, 27] focusing only on the effects of binary mixtures. Tajima *et al.* [28] reported a statistically designed experiment to screen the interactions of five *Fusarium* mycotoxins. They also found interactions with the mycotoxins studied and concluded, that the effect of the mycotoxin mixture cannot be predicted solely on the basis of the effect of the individual compounds. Langseth *et al.* [29] studied the cytotoxicity of Norwegian grain extracts but did not find any correlation between the trichothecene concentration and toxicity of the samples. However, they found a positive correlation between *Fusarium avenaceum* contamination of the samples and the cytotoxicity, suggesting that the secondary metabolites produced by *F. avenaceum* were responsible for the toxic effects. In our study, the concentrations of mycotoxins produced by *F. avenaceum* (BEA, ENNs, MON) were analyzed, but possibly due to the very low contamination levels, no correlation with the cytotoxic response could be found.

The calculated cytotoxic response proved to be a suitable measure for the cytotoxicity of the samples comprising complex mixtures. Although, the concentrations of 16 different mycotoxins were determined in the samples, other unidentified secondary metabolites or sample components may still be present and cause toxic effects to the cell line and, in these cases, the use of a top-down model is justified. The fact that all samples evoked cytotoxicity for FL cells, but that no correlation with the mycotoxins could be found, indicates that the cell line used was more sensitive and more specific to other compounds than to the mycotoxins – at least for these mycotoxin concentrations, and so the cytotoxicity evoked by the mycotoxins might have been covered by these other components of the samples. The cytotoxicity evoked by the spiked samples, however, shows that if only the concentrations are high enough, the FL cells can be used for the measurement of cytotoxicity. Although several cell lines were tested for their sensitivity, further studies on other cell lines are still needed to find a more sensitive and more specific cell line for the measurement of cytotoxicity for sample extracts with low levels of mycotoxins. In most cases, a single mycotoxin has been used to induce cytotoxicity and an EC_{50}-value (concentration evoking 50% inhibition of growth) reported [13]. However, some attempts have also been made using crude extracts to measure toxic effects on the cell lines [29].

4 Concluding remarks

This study demonstrates that the mycotoxins analyzed do not pose any obvious health risk in Finland or Italy. However, it must be noted that this study only reflects the situation of the products on the market during the spring of 2002. Changes in the growing conditions for the crops may promote the toxin production of the fungi and the concentrations may vary greatly between different years of harvest. On the other hand, the *in vitro* tests showed that the grain-based products may have cytotoxic effects, though those seem to be attributable to compounds other than mycotoxins.

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