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► To cite this version:

Arpad Ambrus, Maria Szeitzne-Szabo, Andrea Zentai, Judit Sali, Istvan Jozsef Szabo. Exposure of consumers to deoxynivalenol from consumption of white bread in Hungary. Food Additives and Contaminants, 2011, 28 (2), pp.209. 10.1080/19440049.2010.540720 . hal-00660889

HAL Id: hal-00660889

<https://hal.science/hal-00660889>

Submitted on 18 Jan 2012

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Journal:	<i>Food Additives and Contaminants</i>
Manuscript ID:	TFAC-2010-215.R1
Manuscript Type:	Original Research Paper
Date Submitted by the Author:	25-Oct-2010
Complete List of Authors:	Ambrus, Arpad; Hungarian Food Safety Office Szeitzne-Szabo, Maria; Hungarian Food Safety Office Zentai, Andrea; Hungarian Food Safety Office Sali, Judit; Hungarian Food Safety Office Szabo, Istvan; Hungarian Food Safety Office
Methods/Techniques:	Exposure - prob modelling, Exposure assessment
Additives/Contaminants:	Mycotoxins - trichothecenes
Food Types:	Cereals

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Manuscripts

Exposure of consumers to deoxynivalenol from consumption of white bread in Hungary

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Abstract

In view of the frequent occurrence of some mycotoxins in cereals, a study was initiated to obtain information for assessing the exposure of the Hungarian adult population. The consumption figures of 1360 individuals based on 3-day record questionnaire indicated that white bread amounted to the major proportion of intake of cereal-based products. Various cereal products were analysed for 16 mycotoxins by a LC/MS/MS multi-toxin method with LOD of 16 µg/kg and LOQ of 50 µg/kg. Deoxynivalenol (DON) was most frequently detected, but no acetyl-deoxynivalenol was present in detectable concentrations. Consumer exposure was calculated with standard Monte Carlo probabilistic modelling and point estimates, taking into account bread consumption and DON contamination in independently taken wheat flour and wheat grain samples. Over 55% of cases the DON intake was below 15% of the PMTDI of 1 µg/kg bw/day. However, in about 5-15% of cases the intake from bread consumption alone exceeded the PMTDI. The wheat grain data led to the higher percentage. The intakes estimated from both data sets were at or below the ARfD of 8 µg/kg bw/day in 99.94%-99.97% of cases.

Keywords: *Cereals DON contamination, probabilistic modelling, exposure assessment, consumer protection*

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1 Introduction

Unfavourable weather conditions may lead to severe *Fusarium* infections of cereals in Europe and other parts of the world, and consequently high level of fusarium toxins. Over 300 mycotoxin-type substances have been identified, as secondary metabolites of *Fusarium* species. The trichothecenes [e.g. deoxynivalenol (DON), T-2 and HT-2 toxin, nivalenol, 4-acetyl nivalenol (fusarenone X)] zearalenon (F-2 toxin), and fumonisins are considered the most important in Europe. DON may be present together with 3-acetyl-DON and 15-acetyl-DON, as well as less frequently with nivalenol (Larsen et al, 2004). This can induce an additive effect when the negative toxic actions of individual mycotoxins increase (Speijers and Speijers 2004).

Symptoms of acute DON intoxication described in human patients include abdominal pain or a feeling of fullness in the abdomen, dizziness, headache, throat irritation, nausea, vomiting, diarrhoea, and blood in the stool (Peraica et al. 1999). The European Scientific Committee on Food derived a tolerable daily intake of 1 µg/kg bw/day (European Commission 1999) for DON. The general toxicity and the immunotoxicity of DON were considered to be the critical effects. In 2010, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) established a group provisional maximum tolerable daily intake (PTMDI) of 1 µg/kg bw for DON and its acetylated derivatives, and also a group acute reference dose (ARfD) of 8 µg/kg bw for DON and its acetylated derivatives (FAO/WHO 2010). The maximum permitted levels of DON (ML) set by the European Commission are 1250 µg/kg for wheat and 750 µg/kg for wheat flour (European Commission 2006)..

DON is highly water soluble, stable under mild acidic conditions but unstable in alkali. During baking and cooking it is stable at 120 °C, moderately stable at 180 °C and partially stable at 210 °C (WHO 2001). These properties affect its processing fate (Samar et al. 2003). The pattern of *Fusarium* infection in the kernels is crucial to the subsequent fate of the toxins during processing (Scott et al. 1984). Nowicki et al (1988) reported that the distribution of DON into the milled wheat fractions was dependent on the degree of fungal penetration into the endosperm of the wheat kernel, which was affected by the wheat variety. The most highly contaminated fractions are those that contain the whole or the outer portions of the grain. Consequently, the DON content may depend on the grade of flour (Hazel and Patel 2004).

The processes used for baking bread and non-yeasted products (cake/biscuits) vary considerably throughout the world. Differences in fermentation and baking conditions (time, temperatures and additives in the dough mixture) have varying effects on DON levels in final

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baked goods. DON remained mostly unaffected during making bread, cakes and biscuits. The reduction of DON during cake manufacture was approximately inversely proportional to the flour content of the product, indicating that DON was practically stable during these processes (Scudamore et al. 2009). Processing including extrusion at temperatures greater than 150 °C resulted in medium to low reduction of DON (Neira et al. 1997).

In Europe, the level of mycotoxin contamination varies depending on agricultural practice and weather conditions. The comprehensive evaluation of the results of surveys reported from 12 EU Member States indicated that 61% of the 6358 wheat samples contained DON in the range of 2 µg/kg (LOD) to 50000 µg/kg (European Commission 2003). The average total DON intake [$\Sigma(\text{mean daily food consumption} \times \text{adjusted mean DON concentration})$] reported from 12 countries ranged from 14.45%-46.1% and 11.3%-95.9% of the 1 µg/kg bw TDI for adults and for infants, respectively. Where no DON was present in detectable concentration, the adjusted mean concentration was calculated as LOD/2 if numerical values between limit of detection (LOD) and limit of quantification (LOQ) were available. In other cases the non-detected contamination (below LOQ) was replaced with LOQ/6. The high level intake, calculated as $\Sigma(95^{\text{th}} \text{ percentile food consumption} \times \text{adjusted mean occurrence data})$, was close or exceeded the TDI in some countries, especially for young children and infants. Similar results were obtained in other studies as well (Larsen et al. 2004). The contribution of wheat, wheat flour or bread consumption amounted to about 76-90% of DON intake in 6 countries (European Commission 2003). The DON concentration reported from more recent years from other European countries are also in the same range (Šliková et al. 2008; Polišenská et al. 2008).

In view of the potentially high level of exposure revealed by the European survey and the mycotoxin contamination level reported more recently from Hungary and some other countries, the Hungarian Food Safety Office (HFSO) initiated a survey of mycotoxin contamination of various cereal based products in order to obtain sufficient information on the exposure of Hungarian consumers. In addition to the data originated from this survey, the data of flour analyses from the regular monitoring programme was also included. Further on, data from pre-marketing control of wheat performed by the official control bodies were also evaluated separately. The results of the survey and monitoring programmes indicated that the most frequently occurring mycotoxin in cereal products is DON. The low frequency of occurrence of other toxins indicated no intake problem derived from cereal products. The

1 results of the exposure assessment based on the DON contamination of wheat flour and
2 unprocessed wheat samples, taken independently, are presented in this paper.

3 4 **Materials and methods**

5 The sampling programme for the HFSO survey, implemented by official inspectors,
6 comprised of seven food items (wheat flour, wheat bran, graham flour, spelt wheat flour, oat
7 bran, rye flour and semolina). As wholemeal bread is very rarely consumed in Hungary,
8 wholemeal bread and wholemeal flour were not included in the sampling programme.
9 The number of lots to be sampled was distributed among the 19 counties taking into account
10 the scale of production. Thus the samples, derived from the 2007 and 2008 production in
11 approximately equal proportion represented the whole country. Wheat samples were taken as
12 part of the regular control of cereal products before marketing. Altogether, analytical results
13 of 176 wheat flour and 147 unprocessed wheat grains sample were available for this study.

14 A multi-toxin method (Sulyok et al. 2006) was adapted and optimised for an Agilent
15 QQQ 6410 LC-MS/MS equipped with positive and negative APCI ion source. The optimised
16 method was subsequently validated for the analyses of acetyl-deoxynivalenol (AcDON),
17 aflatoxin B1, aflatoxin B2, aflatoxin G1, aflatoxin G2, deoxynivalenol (DON), diacetoxysc
18 scirpenol, fumonisin B1, fumonisin B2, fusarenon-x ,nivalenol, neosolanid, ochratoxin A,
19 HT-2, T-2, and zearalenone in cereal products. The typical performance parameters for DON
20 determination were: recovery 85-120%, limit of detection (LOD) 16 µg/kg, and limit of
21 quantification (LOQ) 50 µg/kg. The reproducibility of the method, expressed as the relative
22 standard deviation, was around 15% at 110 µg/kg concentration.

23 The analyses of samples containing DON above the maximum permitted level were
24 repeated with the accredited individual HPLC based methods of the laboratory. The results
25 were not significantly different and confirmed that the multi-toxin method provided similar
26 results as the routinely used compound specific methods. The DON contamination of wheat
27 samples was determined with ELISA methods (Euro-Diagnostica 1999; VICAM 1999)
28 having an LOQ of 100 µg/kg. The results obtained with the HPLC and ELISA methods at
29 around the permitted maximum concentration were comparable taking into account their
30 uncertainties.

31 The food consumption figures were obtained from a national nutritional survey carried
32 out in 2003-04, which was based on the 3-day record questionnaire including food
33 consumption of two non-consecutive working days (choosing the days freely) and of Saturday

1 or Sunday (Rodler et al. 2005). The database comprised of the validated consumption figures
2 of 1360 adults and provided information on sex, age, and body weight of the individuals. For
3 calculation of consumer exposure, the three-day average consumptions were divided by the
4 body weight of the consumer. The few missing body weight data were replaced by the
5 average body weight of 81.4 kg for male and 68.1 kg for female consumers. The non-
6 consumers were counted with zero consumption. As only 10 consumers of 1360 did not eat
7 bread, the no-consumption days had no effect on the calculation of the mean, 95th or 97.5th
8 percentiles of the consumption. The consumption figures indicated that the vast majority of
9 the cereal based food derived from wheat flour, and the white bread consumption amounted to
10 its major portion. The empirical relative and cumulative frequency distributions of white
11 bread consumption is shown in Figure 1.

12 The DON concentration in bread may be affected by milling of wheat and making
13 bread from flour. The effect of processing is expressed with the processing factor, which is
14 defined as the ratio of the concentrations of the substance examined (C_{proc}) in the processed
15 product and the raw agriculture commodity (C_{Raw}) (FAO 2009a).

$$Pf = \frac{C_{Proc}}{C_{Raw}}$$

17 As several factors are affecting the natural DON content of wheat flour, the reported
18 processing factors vary to a large extent: 0.326, 0.365, 0.419, 0.471, 0.516, 0.534 and 0.653
19 (Hazel and Patel 2004; Visconti et al. 2004; Rios et al. 2009). The calculated median and
20 mean processing factors were 0.471 and 0.469, respectively.

21 The median processing factor (P_{f2}) of 0.471 was applied in this study to convert DON
22 concentration from wheat to flour following the practice of the FAO/WHO Expert Panel on
23 Pesticide Residues, JMPR (FAO 2009b). As in this case the median and the mean processing
24 factors were very similar either of the values could have been used.

25 The DON concentration in bread was calculated based on the typical recipe using 700 g flour
26 for 1 kg bread ($P_{f1}=0.7$) and assuming that the DON concentration did not decrease during
27 baking.

$$C_{DON,bread} = C_{DON\ flour} \times P_{f1} = C_{DON,flour} \times 0.7$$

29 Thus, the DON concentration in bread ($C_{DON,bread}$) was calculated from the measured values in
30 wheat ($C_{DON,wheat}$) using the median of processing factors published in the scientific literature
31 as:

$$C_{\text{DON,bread}} = C_{\text{DON,wheat}} \times P_{f1} \times P_{f2} = C_{\text{DON,wheat}} \times 0.7 \times 0.471$$

As the consumption figures did not distinguish the large variety of breads produced in Hungary, we assumed that the different quality grades of white flour sampled were all used to make bread.

The DON concentrations below LOD or LOQ were taken into account in two ways for calculation of consumer exposure:

- (a) If the reported actual LOQ or LOD where available, the reported values were used for the calculation of the mean intake;
- (b) If both LOD and LOQ were available, mean level were calculated using LOD/2 for results lower than the LOD. For results between LOD and LOQ, numerical values, if available, were used. If only LOQ was available, the LOQ/6 was used for values below the LOQ.

The sampling of wheat and wheat milled products were carried out completely independently, therefore the DON contamination in wheat and wheat has no direct relationship. Consequently, the DON intake through bread was calculated from the DON concentrations measured in flour samples and wheat samples separately. The two independent data sets provided two different estimates for the intakes.

The probability distribution of consumer exposure in $\mu\text{g/kg bw/day}$ was estimated by two procedures:

Procedure A: multiplying the bread consumption ($\text{kg bread/kg bw/day}$) with the DON concentration in bread ($\mu\text{g/kg}$) after drawing 200 000 random samples with replacement from each of the populations of bread consumption and the calculated DON concentrations in bread from the measured concentrations in flour or wheat using the processing factors described above. This method is a standard Monte Carlo technique that resamples points from the consumption and concentration data.

Procedure B: multiplying all consumption figures with all DON concentrations derived from wheat data, resulting in 199920 (1360×147) intake values. As in the latter case all possible product combinations are used once, calculated figures provide the best estimate for the intake distribution, which can be used to assess the applicability of procedure 'A'.

1 Both methods should approximate the true underlying intake distribution if the original data
2 has large sample sizes. The number of samples was limited in our study assuring to find at
3 least one value above the 98th percentile of the DON concentration present in the flour
4 samples with 95% probability only. Consequently, the presence of higher DON concentration
5 in the marketed wheat flour than seen in the samples analysed cannot be excluded.
6
7 The effect of selection of lowest concentration was studied by performing the calculations
8 with the procedures described above.

9 **Results and discussion**

10
11 The number of samples analysed within the HFSO programme together with the
12 concentration ranges of mycotoxins detected are summarised in Table 1. There were 144
13 various grades of wheat flour samples. Further on, the results of the analyses of 32 wheat
14 flour samples were available from the regular random monitoring programme in Hungary. In
15 the latter case the LOD was not reported, and the limit of quantification of DON varied
16 between 25 and 222 µg/kg. The acetylated derivatives of DON were not present in detectable
17 amounts in any of the samples.

18 In addition to the HFSO survey, the results of wheat sample analyses carried out in
19 Hungary by three organisations (CAO, CC and W) in 2008 were made available for the
20 evaluation of DON contamination. The summary statistics of the DON concentrations in
21 various samples are shown in Table 2. The Mann-Whitney U-test revealed no significant
22 differences between the two data populations obtained by CC and W. The DON measured in
23 wheat by CAO was in the middle of the concentration range. Consequently the three DON
24 populations in wheat grains were combined and the 147 results were also used for assessing
25 the consumers' dietary exposure.

26 The estimated relative and cumulative frequency distributions of DON contaminations
27 in white wheat flour and wheat grains are presented in Figure 2. The nature of the distribution
28 of DON in flour and wheat grain samples is similar indicating that the high values are
29 occurring with very low frequency. The heavy tail of DON distribution in wheat might
30 indicate that much larger DON values are possible, beyond those seen in the data. The relative
31 frequency distributions of DON daily intake based on the independent 176 flour and 147
32 wheat grain samples are shown in Figure 3. The most frequent intake calculated from DON

contamination of flour is lower than that derived from wheat grains (around 0.05 and 0.1 $\mu\text{g/kg bw/day}$, respectively), but the frequency of higher intakes becomes very similar.

Some selected parts of the cumulative intakes calculated are summarised in Table 3. The table includes the calculations carried out with original data sets using the reported LOQ or LOD values for non-detected DON contamination. In addition, the intake calculations were also performed with the LOQ/6 or LOD/2 values where the latter ones were available.

The results in Table 3 show that in over 55% of the cases the DON intake of the Hungarian adult population is below 15% of the PMTDI of 1 $\mu\text{g/kg bw/day}$. However, in about 5-15% of the cases the intake from bread consumption alone was at or above the PMTDI. The intake was at or below the ARfD of 8 $\mu\text{g/kg bw/day}$ in 99.94-99.97 of cases.

The intake figures obtained with DON data adjusted for the non-detected contamination showed similar tendency. The intake estimates based on wheat flour indicated that 50% of the cases the intake was at or below 9% and 12% of PMTDI. The intake figures calculated with adjusted DON concentrations were somewhat lower than those obtained from non-adjusted data, as expected. The probability distribution of DON intake was not affected by the LOQ of the analytical methods at or above the PMTDI.

The exposure exceeding the PMTDI occurred, notwithstanding that only 2% of the flour samples contained DON above the 750 $\mu\text{g/kg}$ maximum limit permitted by the EU legislation. In case of wheat grain, the DON contamination was above the ML of 1250 $\mu\text{g/kg}$ in 20% of samples, which resulted in higher probability of exposure over the PMTDI.

The intake distributions calculated with procedures A and B gave practically the same results as shown in the respective columns in Table 3. The repeatability of intakes calculations based on drawing 200000 random samples with replacement (DON in white flour RND1 and RND2) was very good. Consequently, the calculation of the probability distribution based on 200000 random samples is sufficiently precise. Comparison of the values obtained with random sampling and with multiplying all DON figures with all consumption data indicates that the method of calculation did not affect the cumulative intake distribution.

In order to enable comparison of our intake estimates with previously published results, the DON intake was also calculated with point estimates summarised in Tables 4 and 5. The exposure assessment with multiplying the medians of consumption and DON contamination corresponded well with the estimates obtained with probabilistic modelling

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1 using both wheat flour and wheat grain DON data. The calculated exposure of high
2 consumers [95th (European Commission 2003) and 97.5th percentile (WHO 2010)] assuming
3 average DON contamination in wheat flour was below 60% of PMTDI, while the calculations
4 based on wheat DON contamination indicated an exposure at the PMTDI.

5
6 **Conclusions**

7 The results indicate that the exposure of adult Hungarian population to DON from the
8 consumption of white flour based bread alone exceeded the provisional maximum tolerable
9 daily intake of 1 µg/kg in 5- 15% of the cases during the period of 2008-2009. Though the
10 white bread is a substantial portion of the wheat flour based food, the additional sources of
11 DON intake will further increase the exposure level.

12 The intake was at or below the acute reference dose (ARfD) of 8 µg/kg bw/day in
13 99.94-99.97 of cases. The low frequency of occurrence of high intake shows that it may be
14 considered as one day event and the intake figures should not be compared to PMTDI. They
15 may only cause acute intake concern. The use of 3-day average consumption figures would
16 reduce the estimated short term intake in most cases. However, the daily bread consumption
17 was very similar for the individual consumers, and the estimated high intake figures were
18 unlikely affected significantly by the average consumption values.

19 The cumulative frequency distributions derived from DON contamination in two
20 independently taken sets of random samples of wheat grain and wheat flour provided two
21 estimates for the intake. There was a difference in the intake at around the PMTDI level. The
22 point estimates based on wheat grain data also indicated substantially higher exposure than
23 those obtained from wheat flour. The differences might be attributed to the limited number of
24 samples amounting to the two data sets. Higher number of samples would be desirable for
25 obtaining more precise intake estimates. Alternatively, a more refined probabilistic methods
26 such as fitting parametric distribution to the data could be used to account for this sampling
27 uncertainty. The method of taking into account the DON contamination below the limit of
28 quantification did not affect the frequency of occurrence of high exposure level.

Acknowledgement

The valuable cooperation and support of Ádám Tölgyesi, Edith Szabó, Géza Muranszky Judit Schill, and Tamás Szigeti are highly appreciated.

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For Peer Review Only

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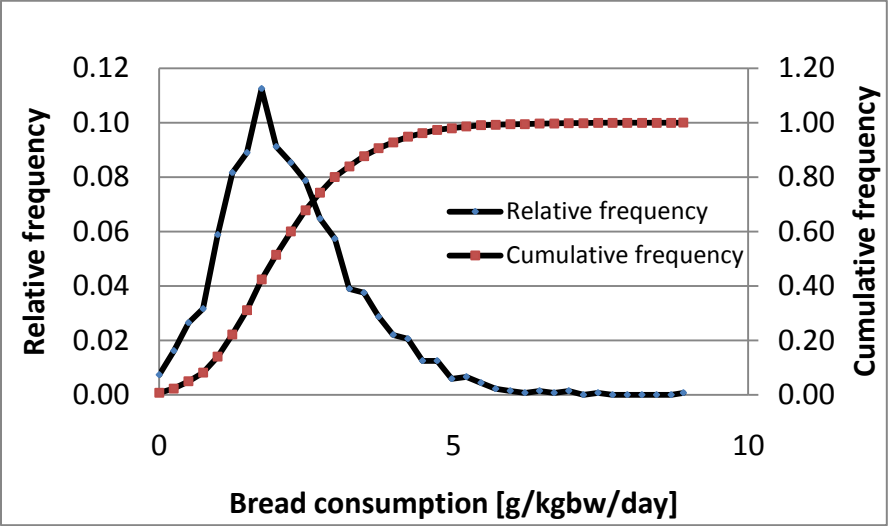


Figure 1. Relative and cumulative frequency distribution of bread consumption.

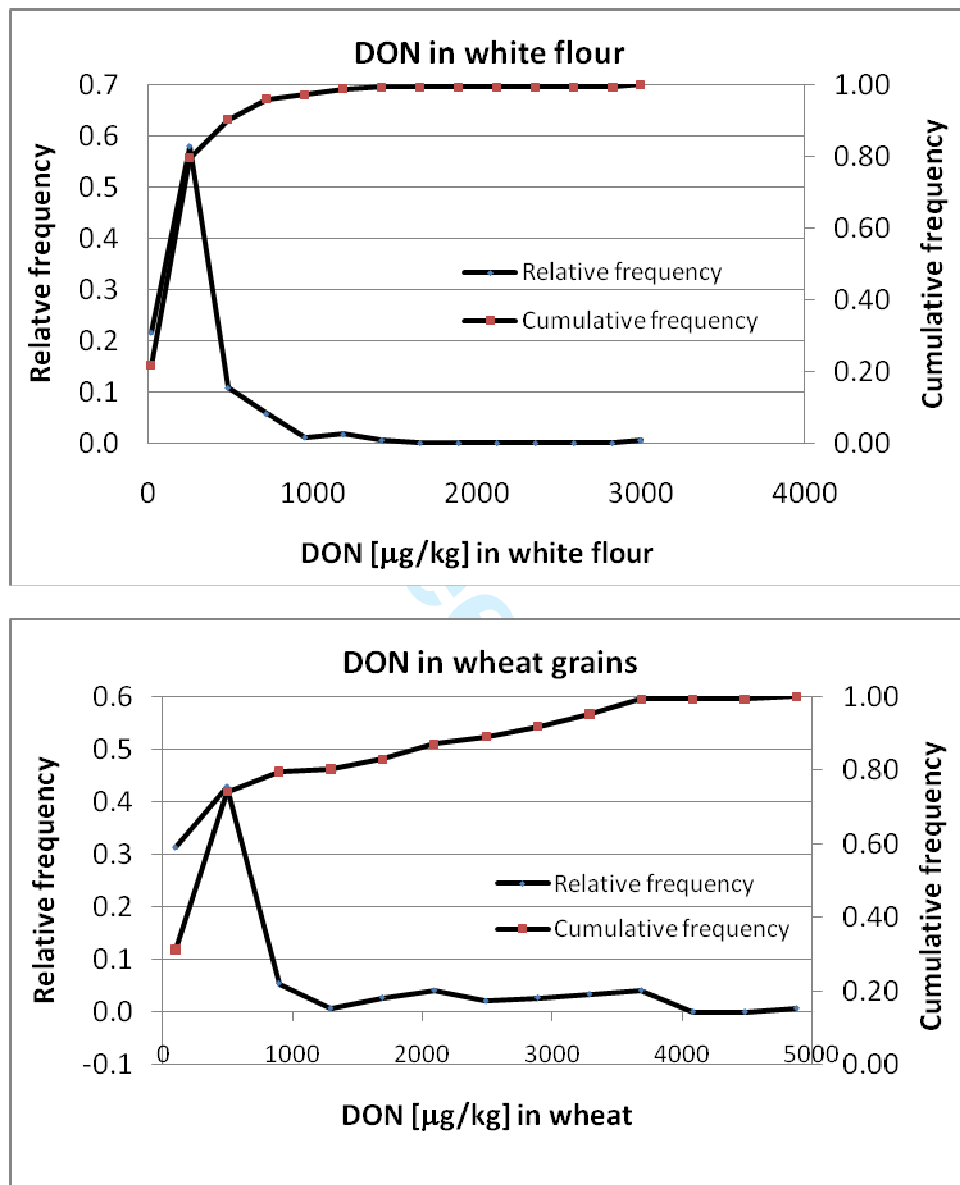


Figure 2. Relative and cumulative frequency distribution of DON in white wheat flour and wheat grains and.

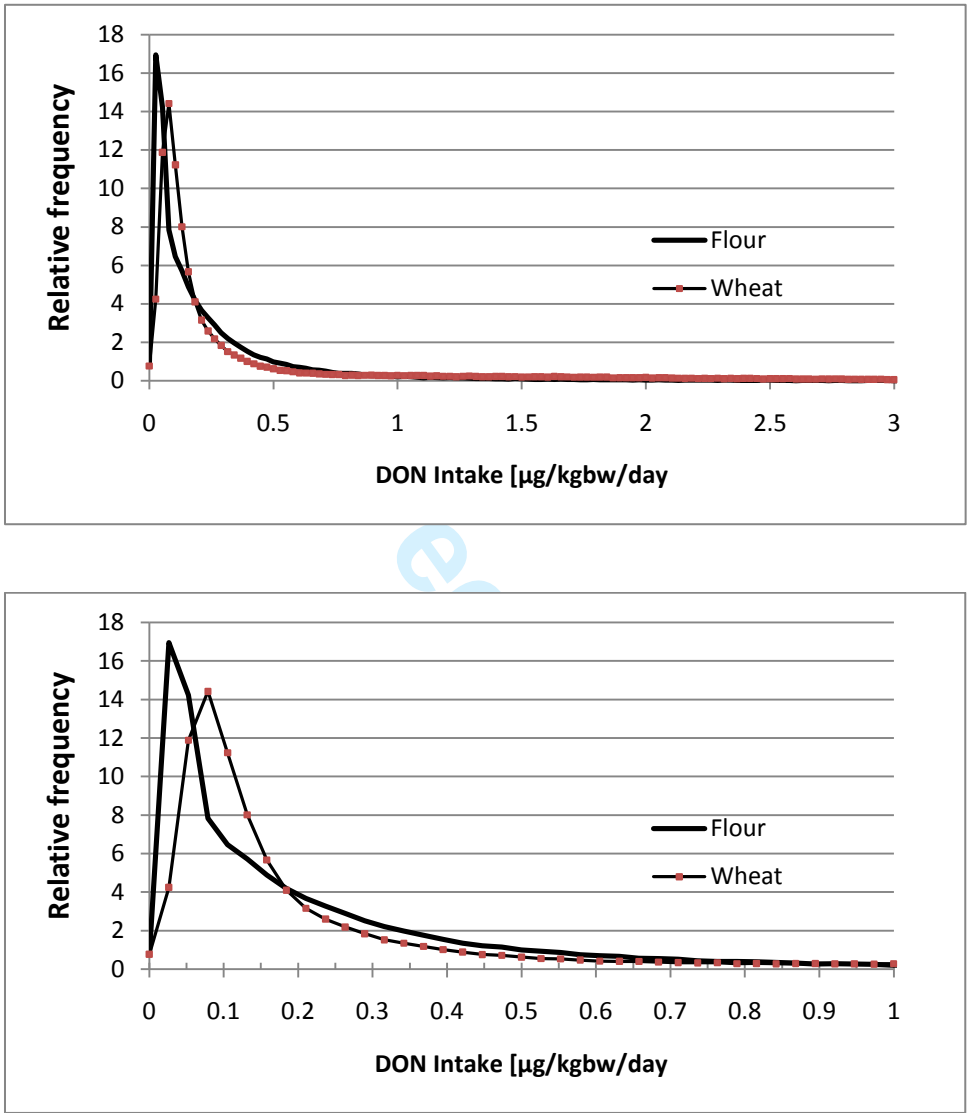


Figure 3. Selected parts of the relative frequency distribution of daily intake of DON ($\mu\text{g/kg bw/day}$) from consumption of white bread calculated from the DON contamination of wheat flour and wheat grains measured in independent random samples. The lower figure shows the intake range up to 1 $\mu\text{g/kg bw/day}$.

Table 1: Summary of cereal products analysed for DON

Product analysed	Sample number	LOD (µg/kg)	DON concentration [µg/kg]			Samples with ²	
			Minimum	Mean ¹	Maximum	R>ML pcs	R<LOD or LOQ [%]
Wheat flour ³	134	16	<16	186.6	3065	7	30.7
Wheat bran	20	16	<16	303.1	1308	2	10.0
Graham flour	5	16	<16	202.6	981	1	80.0
Rye flour	26	16	<16	58.0	375	0	69.2
Spelt wheat flour ³	10	16	<16	91.0	625	0	60.0
Oat bran	15	16	<16	13.9	97	0	93.3
Semolina	6	16	<16	104.7	428	0	50.0
Wheat flour ⁴	32	25-222 ⁵	< 25	129.8	1065	1	59.4

Notes: 1: The average concentration was calculated with LOQ/6 and LOD/2 where non-detectable residues were reported

2: R>ML pcs indicates the number of samples containing DON above maximum legal limit. R<LOD or LOQ indicates non-detected DON.

3: DON concentrations in wheat and spelt wheat flour were considered together

4: Samples taken within the regular programme of CAO

5: LOQ values were only reported

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Table 2: Descriptive statistical parameters of DON [$\mu\text{g/kg}$] in wheat and wheat flour samples

	DON in wheat grains					DON in wheat flour	
	CC	W	CAO	Combined CC+W+CAO	Combined LOQ/6	Original data	LOD/2 or LOQ/6
No<LOQ/LOD	27	11	0			57	
Min	100	100	100	100	16.7	16	8
Median	201	119		200	200	101.5	71.5
Mean	775.1 ^a	376.5 ^a		700.3 ^a	674.8	189.8	174.2
P _{0.975}	3574	1876		3499	3499	933	933
Max	4881	1920	825	4881	4881	3065	3065
SD	1136	521.3	643 ^b	1056	1071	308.48	312.63
CV	1.47	1.38	1.39	1.51	1.59	1.63	1.79
Count	119	26	2	147	147	176	176

Notes: ^a: calculated with reported LOQ values
^b: calculated with range statistics with reported LOQ.
P_{0.975}: the 97.5th percentile of the DON concentrations calculated with Excel 2007
CC, W and CAO indicate the samples taken in Hungary as part of different control programmes during 2008.

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Table 3. Cumulative frequency of daily intake calculated from DON contamination in wheat grains and white wheat flour

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Intake $\mu\text{g/kg}$ bw/day	Estimates from DON in wheat grains			Estimates from DON in wheat flour ¹		
	Original Proc. A	LOQ/6 Proc. A	LOQ/6 Proc. B	LOQ/6 LOD/2	Original RND1	Original RND2
0	0.77%	0.77%	0.74%	0.77%	0.77%	0.67%
0.05	15.34%	35.92%	35.85%	37.95%	30.95%	30.84%
0.10	40.72%	46.62%	46.59%	52.68%	45.04%	44.94%
0.15	54.65%	55.94%	55.89%	62.08%	55.44%	55.38%
0.20	62.35%	62.62%	62.63%	68.68%	63.26%	63.22%
0.30	70.73%	70.85%	70.87%	77.48%	74.30%	74.23%
0.40	75.35%	75.50%	75.46%	82.92%	81.15%	81.04%
0.50	78.14%	78.28%	78.25%	86.54%	85.53%	85.48%
0.60	80.01%	80.12%	80.14%	89.15%	88.61%	88.57%
0.70	81.46%	81.55%	81.56%	91.13%	90.84%	90.79%
0.80	82.65%	82.74%	82.76%	92.54%	92.39%	92.34%
0.90	83.74%	83.86%	83.83%	93.73%	93.63%	93.56%
0.95	84.26%	84.37%	84.33%	94.20%	94.11%	94.06%
0.98	84.57%	84.67%	84.63%	94.45%	94.39%	94.35%
1.00	84.75%	84.84%	84.81%	94.58%	94.52%	94.50%
1.02	84.97%	85.08%	85.05%	94.76%	94.73%	94.70%
1.05	85.28%	85.38%	85.36%	95.00%	94.98%	94.95%
1.60	90.10%	90.03%	90.11%	97.68%	97.66%	97.67%
1.95	92.53%	92.49%	92.55%	98.45%	98.44%	98.44%
1.98	92.72%	92.69%	92.73%	98.51%	98.50%	98.50%
2.00	92.85%	92.79%	92.85%	98.54%	98.53%	98.53%
2.02	92.96%	92.90%	92.96%	98.56%	98.56%	98.55%
2.05	93.14%	93.07%	93.14%	98.61%	98.62%	98.60%
2.10	93.43%	93.34%	93.41%	98.67%	98.70%	98.67%
3.00	96.98%	96.99%	96.98%	99.38%	99.39%	99.36%
4.98	99.54%	99.55%	99.55%	99.77%	99.78%	99.78%
5.00	99.55%	99.55%	99.56%	99.78%	99.78%	99.79%
5.02	99.56%	99.56%	99.56%	99.78%	99.78%	99.79%
5.05	99.57%	99.57%	99.57%	99.78%	99.78%	99.79%
7.90	99.97%	99.97%	99.97%	99.95%	99.94%	99.94%
7.95	99.97%	99.97%	99.97%	99.95%	99.94%	99.94%
7.98	99.97%	99.97%	99.97%	99.96%	99.94%	99.94%
8.00	99.97%	99.97%	99.97%	99.96%	99.94%	99.94%
8.02	99.97%	99.97%	99.97%	99.96%	99.94%	99.94%
8.05	99.97%	99.98%	99.98%	99.96%	99.94%	99.94%
9.98	99.99%	99.997%	99.99%	99.99%	99.98%	99.98%
10.00	99.99%	99.997%	99.99%	99.99%	99.99%	99.98%
10.02	99.99%	99.997%	99.99%	99.99%	99.99%	99.98%
10.61	99.997%	99.999%	100.00%	99.99%	99.99%	99.99%
≥ 11.76	100.00%	100.000%	100.00%	100.00%	100.00%	100.00%

1. The maximum exposure calculated was 15.68 $\mu\text{g/kg}$ bw/day. Intakes were calculated with procedure A.

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Original: the non-detected DON concentrations were taken as LOQ or LOD from the reported results.

RND1 and RND2: drawing random numbers with replacement was repeated twice.

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Table 4: Exposure of consumers¹ [$\mu\text{g/kg bw/day}$] from bread calculated from DON contamination in wheat flour

White bread consumption g/kg bw/day		DON concentration [$\mu\text{g/kg}$] ²			
		Median	Mean	P0.95	P0.975
		71.05	132.8	454.65	652.8
Median	1.94	0.138	0.258	0.882	1.266
Mean	2.12	0.151	0.282 ¹	0.964	1.384
P0.95	4.56	0.324	0.606	2.073	2.977
P0.975	4.78	0.340	0.635	2.173	3.120

White bread consumption g/kg bw/day		DON concentration [$\mu\text{g/kg}$] ³			
		Median	Mean	P0.95	P0.975
		50.05	122	454.65	652.8
Median	1.94	0.097	0.237	0.882	1.266
Mean	2.12	0.106	0.259 ¹	0.964	1.384
P0.95	4.56	0.228	0.556	2.073	2.977
P0.975	4.78	0.239	0.583	2.173	3.120

1. [Exposure of consumers \[\$\mu\text{g/kg bw/day}\$ \] shown in the table were calculated by multiplying the DON concentration with the intake figures \(e.g. mean \$\times\$ mean\)](#)
2. The DON concentration was taken as the reported LOQ or LOD values
3. The DON concentration was calculated as LOQ/6 or LOD/2 from the reported values

Table 5: Exposure of consumers¹ [$\mu\text{g/kg bw/day}$] from bread calculated from DON contamination in wheat grains

White bread consumption g/kg bw/day	DON concentration [$\mu\text{g/kg}$] ²			
	Median	Mean	P0.95	P0.975
	65.94	230.9	1081.25	1153.6
Median 1.94	0.128	0.448	2.098	2.238
Mean 2.12	0.140	0.490 ¹	2.292	2.446
P0.95 4.56	0.301	1.053	4.931	5.260
P0.975 4.78	0.315	1.104	5.168	5.514

White bread consumption g/kg bw/day	DON concentration [$\mu\text{g/kg}$] ³			
	Median	Mean	P0.95	P97,5
	65.94	222.5	1081.25	1153.6
Median 1.94	0.128	0.432	2.098	2.238
Mean 2.12	0.140	0.472 ¹	2.292	2.446
P0.95 4.56	0.301	1.015	4.931	5.260
P0.975 4.78	0.315	1.064	5.168	5.514

1. Exposure of consumers [$\mu\text{g/kg bw/day}$] shown in the table were calculated by multiplying the DON concentration with the intake figures (e.g. mean $\times$ mean)
2. The DON concentration was taken as the reported LOQ or LOD values
3. The DON concentration was calculated as LOQ/6 or LOD/2 from the reported values