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Annual variation of deoxynivalenol in Danish wheat flour 1998 – 2003 and daily intake by the Danish population

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10 **Annual variation of deoxynivalenol in Danish wheat flour 1998 – 2003**
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12 **and daily intake by the Danish population**
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17 Peter Have Rasmussen, Annette Petersen and Faranak Ghorbani,
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Abstract

The occurrence of deoxynivalenol (DON) in Danish wheat flour was studied during the period from 1998 to 2003 by respectively capillary gas chromatography with electron capture detection and liquid chromatography coupled to an ion trap mass spectrophotometer. A total of 151 samples were collected from mills and the retail market in Denmark. The contamination level varied considerably from year-to-year with the highest concentrations occurring in samples from the 2002 harvest with mean and median concentrations of 255 $\mu\text{g}/\text{kg}$ and 300 $\mu\text{g}/\text{kg}$, respectively. Compared to the other harvest years 2002 had the highest amount of precipitation around flowering time in the end of June and until the beginning of July covering the weeks 25-27. The lowest average levels were found in samples from the harvest of 2001, where the weeks 25, 26 and 27 were dry compared with the other harvest years. The highest value (705 $\mu\text{g}/\text{kg}$) was obtained in a flour sample from the 2002 harvest, but none of the tested samples exceeded the maximum limit of 750 $\mu\text{g}/\text{kg}$, which has been recently introduced by the European Commission for DON in flour used as raw materials in food products. Calculation of the chronic or usual intake by a deterministic approach showed that the intake did not exceed the TDI of 1 $\mu\text{g}/\text{kg}$ bw/day either for the whole population or for children. A probabilistic approach also showed that the intake in general was below the TDI, but the intake for children in the 99 % percentile amounted to more than 75% of the TDI. The highest intake is calculated to be 2.5 $\mu\text{g}/\text{kg}$ bw/day.

Keywords: Deoxynivalenol (DON), wheat flour, GC-ECD, LC-MS, annual variation, EU maximum limit, intake calculations.

Introduction

Deoxynivalenol (DON) is produced by different *Fusarium* spp. and predominantly found in grains of wheat, barley, oats, rye and maize grown in the temperate regions of Europe, America and Asia. The susceptibility towards infection with DON producing *Fusarium* spp varies between cereals. In North America (Miller, 1994) and to some degree in Europe (Gareis et al., 1989) wheat and maize have been regarded as the grain species most susceptible to *Fusarium*, whereas a survey from Norway revealed oats to be more contaminated with DON than barley and wheat (Langseth and Elen, 1997). In a recent Danish study (Rasmussen et al., 2003) both a higher incidence and concentration levels of DON were found in wheat than in rye flour, indicating that wheat grown in Denmark is more susceptible than rye towards attack from DON producing *Fusarium* spp. Increased levels of DON in cereal grains are often observed in harvest years with frequent rainfall and high humidity during the flowering period. Variation from year-to-year in contamination level has been described in several studies (Scott, 1997; Langseth and Elen, 1997), indicating that the observation of climate data can be used as a preliminary prediction of contamination levels for a certain field area.

The SCOOP report from the European Commission (2003) shows that the major sources of dietary intake of DON are through products made from cereals, in particular wheat and maize, and especially in infants and young children the dietary intake of DON is often close to the tolerable daily intake (TDI) of 1 µg/kg body weight/day (SCF Scientific Committee on Food, 1999). Similarly, studies from the Netherlands showed that the TDI is easily exceeded in harvest years with a high infection level of *Fusarium*

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4 fungi. Thus, in 1998 and 1999 the intake of DON could have exceeded the TDI for
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7 eighty percent of the 1-year- old children (Pieters et al., 2004).
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11 Based on the results of the SCOOP report and other surveys, in June 2005 the European
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13 Union introduced maximum levels of DON in various cereals and cereal products for
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15 human consumption. The maximum permitted level of DON in cereal flour including
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17 maize flour and maize grits is 750 µg/kg (Commission Regulation (EC) No. 1068/2005
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19 of 6 July 2005), and 200 µg/kg in baby food and processed cereal based food for infants
20
21 and young children. The regulation is valid from 1st July 2006. The present study can be
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23 regarded as a continuation of our earlier studies (Rasmussen et al., 2003), where not
24
25 only DON but also other trichothecenes were analysed in flour of wheat and rye. These
26
27 results showed that the incidence and contamination level of nivalenol, HT-2 toxin and
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29 T-2 toxin in Danish wheat flour were low, and for this reason only DON is used to
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31 study the variation in incidence and content level during the six year survey period
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33 between 1998 and 2003. The data is also used to measure the daily intake of the Danish
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35 population with two approaches, the deterministic method and the probabilistic method
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37 based on the Monte-Carlo Risk Assessment (Boon et al., 2005).
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47 **Materials and methods**

48 *Samples*

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50 Flour samples of common wheat were collected each harvest year between 1998 and
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52 2003 in two parts, one in the period from October to December, the other part from
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54 April to June the following year. Samples from 1998 and 1999 were collected both at
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56 the mills as retail packaged samples and from the Danish retail market with
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4 approximately the same distribution between the two places. The samples from 2000 to
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7 2003 were collected at the retail market all over Denmark. Authorized personnel from
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10 local food control units performed the sampling. Only flour produced at Danish mills
11
12 were used in the present study. The mills were located all over Denmark, three in
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14 Jutland, one on Funen and two on Zealand. In Denmark most of the wheat flour for
15
16 human consumption are produced from domestic grains, and information from Danish
17
18 Industry and the millers show that typically over seventy percent of the grains are from
19
20 Denmark. The rest of the grain is imported mainly from the southern part of Sweden
21
22 and Germany.
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28 Flour samples were collected in retail packaging supplied with production date and
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30 batch number. Samples collected at the mill consisted of 5 to 10 bags of approximately
31
32 two kg and samples obtained at the retail stage consisted of 1 to 4 bags from the same
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34 mill each containing two kg of flour. In the laboratory the samples were pooled and
35
36 fully mixed in the a blending tank to produce one aggregate sample of 1 kg. From the
37
38 aggregate sample 25 gram was taken for analysis. The number of samples colleted from
39
40 each harvest year is shown in Table 1.
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47 The mean temperature and precipitation values of June, July and August recorded each
48
49 year (1998 – 2003) at the meteorological stations in Denmark are shown in Table 2
50
51 (Danish Meteorological Institute). Precipitation and temperature data recorded on a
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53 weekly basis from the end of June to the beginning of July are also shown for each year.
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55 This period corresponding to the weeks 25-27 is in the present study assumed to
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57 represent the approximate flowering time for wheat grown in Denmark, although it is
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4 known that there may have been variations from region to region and from year to year.
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6 A general description of the weather conditions during the grain-growing season for
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8 each year, published by the Danish Agricultural Advisory Service (Landsudvalget for
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10 Planteavl, 1998 – 2003) was also used to evaluate the effect of weather conditions on
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12 DON content in flour.
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15 16 17 18 *Extraction and clean-up* 19

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21 Standards of DON were purchased from Sigma St. Louis, MO, USA. Stock solutions of
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23 200-500 µg /ml were made in acetonitrile and stored at –20°C in portions of 1 ml for 1
24
25 year. Working standards were prepared by dilution of stock solutions with acetonitrile
26
27 and stored at a concentration of 100 µg/ml at –20°C. The exact concentration was
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29 measured by U.V. spectrophotometry at 218 nm using $\epsilon_{218} = 6400 \text{ cm}^{-1}\text{M}^{-1}$ (Widstrand
30
31 and Pettersson, 2001). Working standards that had been stored up to 9 months showed
32
33 no decrease in concentration. Extraction and clean-up were performed in accordance
34
35 with the method described by Rasmussen et al., 2003. Twenty-five grams of ground
36
37 and homogenised sample were extracted with 100 ml acetonitrile/water (84/16, v/v) in a
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39 250-ml flask on a shaker for two hours at room temperature. The extract was filtered
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41 through a cellulose filter, and 5 ml of the extract was slowly pressed through a
42
43 MycoSep 225 column from Romer Lab. (Washington, USA). An additional 5 ml of
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45 acetonitrile/water (84/16) was filled in the culture tube of the MycoSep 225 cartridge
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47 and pressed through the column. The eluates were combined and evaporated to dryness
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49 at 45°C using a gentle flow of nitrogen.
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LC-MS Analysis

Samples from the harvest years 1998-2001 were analysed by capillary gas chromatography with electron capture detection (GC-ECD) by the method previously described (Rasmussen et al., 2003), while samples collected from the harvest years 2002 and 2003 were analysed by LC-MS. Liquid chromatography was performed on a Hewlett Packard Series 1100 HPLC system equipped with the corresponding HP 1100 series pump and autosampler. The column was an X-terra MS C18 (5 μm , 3.0 mm x 250 mm, Waters, Milford, MA, USA) protected by a 3.0 x 20 mm guard column of the same material. Isocratic elution with a flow rate of 300 $\mu\text{l}/\text{min}$ was applied over 15 minutes by using the same mobile phase as Razzazi-Fazelli et al. (1999) consisting of acetonitrile:-methanol:water (9 : 9 : 82, v/v/v) . Injection volume was 10 - 20 μl . The HPLC system was coupled to an ion trap mass spectrometer (LCQ, ThermoFinnigan, San Jose, CA) used in the negative mode employing atmospheric pressure chemical ionisation (APCI (-)). The MS parameters were optimised separately by direct infusion of 10 $\mu\text{g}/\text{ml}$ DON in the mobile phase via a T-piece between the column and the ionisation chamber at a flow rate of 5 $\mu\text{l}/\text{min}$. Mass spectra were registered in the full-scan mode between 200 and 350 Da. The capillary and vaporising temperature were 150 $^{\circ}\text{C}$ and 400 $^{\circ}\text{C}$, respectively, and the capillary voltage was set at -47 V. Nitrogen sheath gas flow was approximately 50 ml/min while the auxillary gas was set at 0. Ionisation current of corona discharge was 5.5 μA . Calibration curves performed in the LC-MS buffer alone or in the presence of matrix showed no significant differences between the measured concentrations of DON in different wheat flour sample. Therefore, calibration curves were made in the LC-MS buffer covering a concentration range between 0.05 $\mu\text{g}/\text{ml}$ and 3 $\mu\text{g}/\text{ml}$ corresponding to 16 μg and 960 μg per kg flour.

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7 *Analytical quality parameters*
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9 The detection limit (signal/noise: 3/1) for DON was the same for both the GC-ECD and
10 the LC-MS method corresponding to 20 µg/kg. When calculating exposure, samples
11 found to contain less than the detection limit were defined as having a concentration of
12 half the detection limit, as suggested by Eriksen and Alexander (1998). In each
13 analytical series, a spiking experiment was performed at levels between 50 – 400 µg/kg
14 using flour samples with DON contents below the detection limit. The mean recoveries
15 and standard deviation for DON analysed by LC-MS was 101% ± 12% (range 80-
16 114%, n = 27). The results were not corrected for recovery. A relative standard
17 deviation of 13% was obtained for 14 analyses of an in-house reference material of
18 durum wheat with a mean content of 1470 µg/kg measured over a period of two years.
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Between 2002 and 2004, the LC-MS method has been evaluated five times in proficiency testing by the FAPAS (Food Analysis Performance Assessment Scheme, Central Science Laboratory, York, UK). The z-scores for contaminated wheat and maize flour were -0.5 (n = 62), 1.4 (n = 64), 0.2 (n = 64), -0.9 (n = 55) and -0.7 (n=73), respectively, where n is the number of participating laboratories. Scores are considered satisfactory if $z \leq |2|$. The GC-ECD method has earlier been tested by this procedure and with satisfactory results (Rasmussen et al., 2003). In addition 10 different samples with DON contents between 20 µg/kg and 1800 µg/kg have been analysed by LC-MS and GC-ECD. All the samples with contents above 550 µg/kg consisted of durum wheat including our in-house reference material mentioned above.

Consumption data and methods for intake calculations

The consumption data used for the intake calculations are based on the nationwide dietary survey conducted in 2000 – 2002 by the Department of Nutrition, Danish Institute for Food and Veterinary Research (Andersen et al. 2005). The survey used a seven-day prospective food record with a pre-coded (semi-closed) questionnaire that included answering categories for the most commonly eaten foods and dishes in the Danish diet. The questionnaire was organised according to a normal daily meal pattern. The body weight and age of the participant was also noted and used in the calculations.

In the present study only data on wheat flour are presented, but through the years of 1998-2001 rye flour was also analysed for DON. It was therefore decided to use data for both rye and wheat in the intake calculations. In the food survey the main consumption of rye and wheat are through bread and therefore a conversion of the contents of DON

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4 in flour to contents in bread has been performed. Bread is a wide group and many
5 different kinds of bread are eaten in Denmark. To simplify the calculations the different
6 groups of bread were merged into two groups, namely “wheat bread” and “rye bread”.
7
8 The content of DON in flour was then converted to contents in bread by using a factor
9 of 0.77 for wheat and 0.62 for rye. These figures were estimated from the content of dry
10 matter, protein and carbohydrates in bread, flour, and grain respectively (Poulsen et al.,
11 2005). Baking or heating generally seems to have little effect on the stability of DON
12 (Pestka and Smolinski, 2005), however some studies resulted in a 0 to 40 % reduction
13 of the content (Eriksen and Alexander, 1998). Other data have shown both increase and
14 decrease in DON levels at dough fermentation and baking (Hazel and Patel, 2004) Due
15 to these uncertainties, loss of DON through processing has not been taken into
16 consideration. Levels below LOD have been set to $\frac{1}{2}$ LOD or 10 $\mu\text{g}/\text{kg}$.
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35 The calculations of the usual intake were performed for both the total population of age
36 4 - 75 years (4068 persons) and for children, age 4-14 years (807 persons). The usual
37 intake is used as a synonymy for the chronic or the long-time intake. Two approaches
38 have been used in the calculations for both consumer groups. The first method is the
39 deterministic approach where the intake is calculated by a point estimate, e.g. only one
40 value is calculated. The usual intake is calculated for the relevant consumer group by
41 multiplying the mean consumption for the consumer group with the mean level of DON
42 for each commodity and the total usual intakes calculated by summing up the individual
43 intakes. In Denmark the mean concentration of mycotoxins is most often used in intake
44 calculations as the best estimate a person will be exposed to during a whole life span
45 (Fromberg et al., 2005). In the SCOOP report from 2003 the mean concentration of
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4 DON is also used for the determination of the intake. However, the median
5 concentration of DON can also be used for this purpose, but to what extent the median
6 will represent a better estimate for the intake calculations is out of the scope of this
7 paper to discuss. The other method is the probabilistic approach, where the total
8 distribution of both the content and consumption are used to derive a distribution of
9 intakes as well as percentiles and uncertainties of the intakes. The calculations have
10 been performed using the “Monte Carlo Risk Assessment” program version 4.0
11 (<http://mcra.rikilt.wur.nl/mcra/>) and is described by Boon et al. (2005). In the program
12 the calculation is performed by multiplying each participant’s intake of respectively
13 wheat bread and rye bread with the obtained average content of DON and then summing
14 up the two intakes to give the intake for each consumer from bread. For the total
15 population this gives about 28.000 intakes including non-eating days which are
16 analysed in the programme with statistical methods for usual intakes (Nusser et al.,
17 1996; Guenther et al., 1997)

40 **Results and discussion**

42 *Analysis of deoxynivalenol by LC-MS*

44 DON was determined by LC-APCI –MS in the negative ion mode as the observed
45 signal/noise ratio was 10 – 15 times higher than in the positive ion mode under the
46 given conditions. The mass spectrum consisted of an intensive M-H – ion at m/z 295
47 corresponding to the deprotonated form of DON (Figure 1). In accordance with the
48 study of Razzazi-Fazeli et al. (1999), the fragment ions at m/z 265 and at 247 appeared
49 in all chromatograms. In this study DON has also been detected in the negative mode
50 by LC-MS-MS through collision induced dissociation in the ion trap (collision energy
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4 24 %) of the parent ion at m/z 295. The most predominating daughter ions were the
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6 same as in the MS mode at m/z 265 and m/z 247, respectively. Although the noise level
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8 was lower in the MS-MS mode than in the MS mode, DON at m/z 295 displayed a
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10 stronger signal (signal/noise) in the full scan mode than could be obtained for the two
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12 daughter ions in the MS-MS mode. Quantification was therefore carried out in the MS
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14 mode at m/z 295 and by using the fragment ions detected at m/z 265 and m/z 247 as
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16 verification. The content of DON determined in parallel by GC-ECD and LC-MS in 10
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18 different flour samples of common wheat and durum wheat is shown in Figure 2. Linear
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20 regression analyses showed a significant correlation ($y = 1.08x - 28$, $r^2 = 0.983$, $p <$
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22 0.005 , x: GC-ECD; y: LC-MS) between the concentration measured by the two
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24 methods, confirming that similar results are obtained by GC-ECD and the LC-MS
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26 method. This assumption is furthermore confirmed by the results obtained in
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28 proficiency testing by FAPAS where the z-scores for both methods showed satisfactory
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30 results.
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37 “(Insert Figure 1 and Figure 2 about here)”
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42 *Annual variation*

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44 During the period from 1998 to 2003 the content of DON in Danish produced wheat
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46 flour varied considerably (Table I). The mean values for the precipitation and
47
48 temperature recorded each year in June, July and August are shown in Table II together
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50 with the precipitation and temperature data approximately covering the last two weeks
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52 of June and the first week of July. This period is suggested to represent the time around
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54 flowering of wheat grown in Denmark, although there may be variations from year to
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56 year and from region to region. Despite Denmark is a small country, some years show
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4 fairly large regional differences in precipitation. In the present study it has unfortunately
5 not been possible to evaluate the importance of such factors because it could not be
6 exactly confirmed from where in the country the different mill samples originate.
7 Moreover, a minor part of the samples produced at Danish mills may contain flour of
8 imported wheat grains from mainly southern Sweden and Germany. In spite of these
9 uncertainties it has been possible with proviso to derive some general indications in
10 relation to the importance of summer weather conditions to the overall DON level in
11 Danish produced wheat flour.
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26 All samples from the 1998 harvest were found to contain DON with relatively high
27 mean and median values of 191 $\mu\text{g}/\text{kg}$ and 140 $\mu\text{g}/\text{kg}$, respectively (Table I). The
28 amount of precipitation in June and July for the whole country were 76 mm and 92 mm,
29 and thus considerably higher than the normal precipitation values registered in Denmark
30 between 1961 and 1990 (Table II). In 1998 there were large amounts of precipitation
31 around the flowering stage in the weeks 25, 26 and 27, conditions known to favour
32 *Fusarium* invasion of the grains. (Sutton, 1982; Teich, 1989). The weeks 25 and 26
33 were relatively cold compared to the other years and below the normal temperature of
34 15.6 °C for July. Moreover, due to low temperatures during the harvest season in
35 August with a mean of 14.6 °C for the entire country which were accompanied by heavy
36 showers in many parts of the country, lodging was especially widespread in winter
37 wheat that year (Landsudvalget for Planteavl, 1998). Such factors may also contribute
38 to increased levels of DON in grains (Langseth and Stabbetorp, 1996).
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Insert Table I about here

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7 In 1999, the amount of precipitation in June was high with a mean value of 120 mm for
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9 the whole country and as shown in Table II, the end of June (week 25) and beginning of
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11 July (week 26) were also relatively wet. Lower amounts of rain were observed in week
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13 27 than in the previous year. The end of July and the beginning of August were warm
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15 and dry but accompanied by many showers later in August, and in 1999 harvest
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17 conditions were especially difficult in many parts of the country. (Landsudvalget for
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19 Planteavl, 1999). These conditions may further explain that the incidence and DON
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21 content in the samples were relatively high and showed similar levels compared to
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23 1998.
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31 During 2000 and 2001, the contamination level of DON continued to decrease with the
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33 lowest values occurring in samples from the 2001 harvest, where the average levels
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35 decreased about 6 times compared to 1998. The incidence of DON was also low in
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37 2001, where DON was found in about 60% of samples tested (Table 1). Weeks 26–27
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39 were dry in both 2000 and 2001 compared to the other harvest years, and also week 25
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41 in 2001. Especially July of 2001 was relatively warm with a mean temperature of 17.5
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43 °C, and this combined with low precipitation resulted in a widespread need for watering
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45 crops on many locations in Denmark (Landsudvalget for Planteavl, 2000 and 2001).
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47 The low contamination level of DON found in samples from these two harvest years
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49 may thus be expected due to the dry weather during the growing season. August of 2001
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51 was very rainy with a mean precipitation of 91 mm and 17 days with rain. Harvest
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53 conditions were difficult and quite exceptionally for Denmark, much grain could not be
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4 harvested at all. However, problems with the presence of *Fusarium* in wheat have not
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6 been observed that year (Landsudvalget for Planteavl, 2001).
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11 Insert Table II about here
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16 During the grain growing season 2002 there was a large amount of precipitation from
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18 the middle of June and throughout the summer (Landudvalget for Planteavl, 2002), and
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20 as shown in Table 2, the weeks 25-27 around flowering had the highest observed mean
21
22 precipitation values. This year the infection level with different *Fusarium* spp. was
23
24 more pronounced than in normal harvest years (Landsudvalget for Planteavl, 2002).
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26 This is in accordance with the findings that the highest mean and median values were
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28 observed for the samples collected from the 2002 harvest although the relative incidence
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30 of positive samples was lower than for other years except 2001. This is due to the fact
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32 that many of the samples from this year contained relatively high DON contents above
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34 250 µg/kg (Table 1). The highest concentration observed was 706 µg/kg. In 2002 there
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36 was large regional differences in precipitation (Landsudvalget for Planteavl, 2002) and
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38 it could well be that samples with high DON contents come mainly from areas with
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40 high precipitation around flowering. A similar hypothesis could also be valid for the
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42 harvest years 1998 and 1999. However, as already mentioned, it is difficult to go into
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44 detail on this topic as more information on sample origin is essential.
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54 Compared to 2002, the contamination level of DON decreased in samples from the
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56 harvest in 2003 with average and median values of 148µg/kg and 151 µg/kg,
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58 respectively. A total of 27 samples were analysed and DON was detected in 26 of the
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4 samples and all samples had contents below 224 µg/kg. In June the mean precipitation
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6 was well above the normal value of 55 mm and July was warm with a day and night
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8 mean temperature of 18.1°C, which is 2.5 °C above the normal temperature. The mean
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10 precipitation for the whole country was low in week 25 whereas week 27 had the
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12 highest observed mean precipitation value of 42 mm. August was dry and relatively
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14 warm.
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21 It should be mentioned that HT-2 toxin and T-2 toxin have been measured in 13 of the
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23 flour wheat samples from the harvest 2003 by a LC-MS method described by Razzazi-
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25 Fazeli et al.(2002). However, all tested samples had contents below the detection limit
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27 corresponding to 20 µg/kg (results not shown).
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33 Several studies have indicated that the contamination level of DON in various cereal
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35 grains frequently is correlated with the amount of precipitation during the growing
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37 season with the lowest contents occurring during dry weather conditions (Muller et al.,
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39 1997; Langseth and Elen, 1997; Scott, 1997). The present results are in accordance with
40
41 these findings. Although the number of investigated harvest years is too small to allow a
42
43 firm statistical analysis on this point, there seems to be some relationship between the
44
45 amount of precipitation in the end of June and beginning of July and the concentration
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47 of DON found in the samples. Thus, the highest mean as well as median concentrations
48
49 were obtained in years with the highest amount of precipitation in the weeks 25, 26 and
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51 27, and the lowest concentrations were found in the years 2000 and 2001 with low
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53 precipitation values around flowering. . The amount of precipitation in August on the
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55 other hand showed no apparent relationship to the contamination level of DON. Thus,
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4 the highest amount of precipitation (91 mm) was observed in 2001, where the
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6 contamination level of DON was low, and in 2003 August was very dry with a mean
7
8 precipitation of 41 mm but with a higher DON contamination level than in 2000 and
9
10 2001. As well as being affected by weather conditions other factors may also have
11
12 contributed to both within and between year variations such as soil treatment, crop
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14 rotation and cultivar differences where some are more sensitive toward *Fusarium*
15
16 infection than others (Jørgensen and Rasmussen, 2002). The contamination level can
17
18 also be affected by the degree of *Fusarium* infection in the previous year (Langseth and
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20 Elen, 1997). However, it is not possible to evaluate the importance of these factors from
21
22 the present data.
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30 The European Commission recently introduced a maximum limit of 750 µg/kg for DON
31
32 in flour used as raw material in food products (Commission regulation (EC) No.
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34 856/2005 of 6. June 2005). As shown in Table I, the mean and the median values for the
35
36 DON content in Danish wheat flour are far below the maximum EU limit. The highest
37
38 value of 705 µg/kg was obtained from the harvest 2002, and thus none of the 151 tested
39
40 samples analysed between 1998 and 2003 exceeded the maximum limit of 750 µg/kg.
41
42 Furthermore, only five samples had DON contents above 500 µg/kg, one sample from
43
44 the harvest 1999 and four samples from the harvest year 2002 Table I. Almost 50
45
46 percent of the tested samples had DON contents below 100 µg/kg, 34 samples between
47
48 101 µg/kg and 250 µg/kg and 25 samples between 251 µg/kg and 500 µg/kg. When
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50 comparing the present results with the EU maximum limits, it must be emphasized that
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52 the sampling procedure used in this study did not fulfil the guidelines for official testing
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4 of DON in the wheat flour samples (Commission Directive 2005/38/EC of 6 June
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7 2005).

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11 *Insert Table III about here*

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16 *Usual intake of DON by the Danish population*

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18 The background information for the intake calculations performed by the deterministic
19 approach is shown in Table III. The intake of DON for both the total population and the
20 children are higher for wheat bread than for rye bread, which is due to a lower content
21 of DON in rye than in wheat flour combined with a higher consumption of wheat bread.
22
23 The intake of DON from bread is calculated to 0.17 µg/kg bw/day for the total
24 population and 0.32 µg/kg bw/day for children. The higher intake for children is due to
25 their lower body weight and their consumption of wheat bread, which is almost the
26 same as for the total population. The TDI for DON is 1 µg/kg bw/day (SCF, 2002), and
27 thus the intake calculated by the deterministic approach will not exceed this value.
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42 Using the median values of DON the intake is calculated to be 0.10 µg/kg bw/day for
43 the total population and 0.18 µg/kg bw/day for children. These values are thus lower
44 than the intakes determined from the mean. Whether the mean or median will represent
45 the best estimate for the level a consumer is exposed to for a whole life span can be
46 discussed. As already mentioned, in Denmark the mean level of mycotoxins is usually
47 used in intake calculations as the best estimate (Fromberg et al., 2005). In the SCOOP
48 project from 2003 the mean was also used for the determination of the intake.
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4 The mean intake and percentiles for the usual intake calculated by the probabilistic
5 approach are shown in Figure 3. The intake for the total population and for children is
6 calculated to 0.20 $\mu\text{g}/\text{kg}$ bw/day and 0.35 $\mu\text{g}/\text{kg}$ bw/day, respectively, which are very
7 similar to the values obtained by the deterministic approach. This reflects that bread is
8 consumed nearly every day and that the majority of the samples have DON contents
9 above the detection limit of the method. The calculations are therefore not very sensitive
10 to extreme intakes. The highest intake is calculated to be 2.5 $\mu\text{g}/\text{kg}$ bw/day. At the 99.90
11 % percentile the intake for children is 0.88 $\mu\text{g}/\text{kg}$ bw/day, and children with an intake
12 higher than 1 $\mu\text{g}/\text{kg}$ bw/day belongs thus to the upper 0.1% percent. When only
13 considering the data from the 2002 harvest with the highest determined content of DON
14 in wheat bread (Table 1), the corresponding intake is calculated to be 0.31 $\mu\text{g}/\text{kg}$ bw/day
15 for the total population, which is about twofold higher than the value obtained for the
16 whole period between 1998 and 2003. This stresses the importance of not only using
17 data from a single year when calculating the intake of DON and as well as other
18 substances with wide annual variation. Using a value of zero instead of $\frac{1}{2}\text{LOD}$ for
19 levels below LOD in the probabilistic approach shows only a minor influence on the
20 intakes, as the mean intake changes to 0.34 $\mu\text{g}/\text{kg}$ bw/day for children and is the same
21 for the total population. This is due to the fact that most of the samples had contents
22 above the detection limit of the method and that samples with high DON contents will
23 be of overall importance for the calculated intakes.

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54 Insert Figure 3 about here
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4 The highest intake of cereal products by the Danish population is from bread of wheat
5 and rye, which contribute more than 80 % of the total intakes (Jørgensen et al., 2001).
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9 Other sources from corn based foods of sweet corn, cornflakes, breakfast cereals of oats,
10 rice and pasta, cakes, biscuits and popcorn (SCOOP, 2003), will also contribute to the
11 intake of DON, and especially children can have a high intake of these products. In the
12 high percentile range above 99 % the intake of wheat and rye bread for children will be
13 about 0.8 $\mu\text{g}/\text{kg bw}/\text{day}$ (Figure 3), and by adding these other sources the TDI value
14 can be exceeded for this group of children.
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26 **Conclusion**

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28 Wheat flour on the Danish market showed a wide annual variation in DON
29 concentration during the period from 1998 to 2003, but none of the tested samples
30 exceeded the maximum EU limit of 750 $\mu\text{g}/\text{kg}$. The highest contamination levels were
31 obtained in harvest years with a relatively high amount of precipitation around
32 flowering in the end of June and beginning of July corresponding to the weeks 25, 26
33 and 27. The wide annual variation in DON contents emphasises the importance of not
34 using a single year for intake calculations. Intake calculations of DON with reference to
35 the consumption of wheat and rye bread show that the mean usual intake determined
36 both by a deterministic and a probabilistic approach are similar corresponding to about
37 0.2 and 0.3 $\mu\text{g}/\text{kg bw}/\text{day}$ for the total population and children, respectively. These
38 products contribute more than 80 % of the total intake of cereals indicating that DON
39 does not represent a general food safety problem in Denmark. However, for children in
40 the high percentile range above 99 % the TDI value of 1 $\mu\text{g}/\text{kg bw}/\text{day}$ can easily be
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4 exceeded by adding the contribution from cereal products other than wheat and rye
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6 bread.
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10 11 12 13 14 **Acknowledgements**

15
16 We wish to thank Henrik Hartkopp and Anders Möller, Department of Nutrition,
17
18 Danish Institute for Food and Veterinary Research for delivering the consumption data
19
20 and compiling the flour data into rye and wheat bread.
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24 25 26 **References**

27
28 Andersen NL, Christensen T, Groth MG, Fagt, S, Bilfotft-Jensen A, Hartkopp H,
29 Hinsch H-J, Matthiessen J, Moeller A, Saxholt E, Trolle E. 2005. The Danes' dietary
30 habits 2000-2002. Main results. In Danish, summary in English (Soeboerg: Danish
31 Institute for Food and Veterinary Research).
32

33
34 Boon PE, Mul A., Voet HVD, Donkersgoed GVD, Brette M, Klaveren JD.2005.
35 Calculations of dietary intake of acrylamide. *Mutation Research* 580: 143-155.
36

37
38 Chelkowski J, editor. 1989. *Fusarium, Mycotoxins, Taxonomy and Pathogenicity.*
39 *Topics in Secondary Metabolites*, vol. 2. Amsterdam: Elsevier.
40

41
42 Eriksen GS, Alexander J. 1998. *Fusarium* toxins in cereals – a risk assessment. Nordic
43 Councils of Ministers, TemaNord.
44

45
46 Eskola M, Parikka P, Rizzo A. 2001. Trichothecenes, ochratoxin A and zearalenone
47 contamination and *Fusarium* infection in Finnish cereal samples in 1998. *Food*
48 *Additives and Contaminants* 18: 707-718.
49

50
51 European Commission 2004. SCOOP task 3.2.10. Collection of occurrence data of
52 *Fusarium* toxins in food and assessment of dietary intake by the population of EU
53 member states. European Commission 2004.
54

55
56 FAO/WHO, 2001. Safety evaluation of certain mycotoxins in food. WHO Food
57 Additive Series, 47 (Geneva: World Health Organization).
58

59
60 Gareis M, Bauer J, Enders C, Gedek B. 1989. Contamination of cereals and feed with
Fusarium mycotoxins in European countries. In: *Fusarium Mycotoxins, Taxonomy and*

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53
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56
57
58
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60

Pathogenicity. Topics in Secondary Metabolites. Chelkowski J, editor. Amsterdam, Elsevier, vol. 2, pp. 441-472.

Guenther PM, Kott PS, Carriquiry AL. 1997. Development of an approach for estimating usual nutrient intake distributions at the population level. *Journal of Nutrition* 127: 1106-1112

Fromberg A, Larsen EH, Hartkopp H, Larsen JC, Grandy K, Jørgensen J, Rasmussen PH, Cederberg T, Christensen T. 2005. Chemical contaminants, Food monitoring, 1998-2003. Part 1. Danish Veterinary and Food Administration. Mørkhøj, Denmark.

Hazel CM, Patel S. 2004. Influence of processing on trichotecene levels. *Toxicological Letters* 153: 51-59. Jørgensen K, Larsen EH, Petersen A, Lund KA, Hilbert G, Andersen NL, Hallas-Møller T, Larsen JC. 2001. Chemical Contaminants, Food monitoring, 1993-1997. Part 2. Danish Veterinary and Food Administration, Mørkhøj, Denmark.

Jørgensen LN, Rasmussen PH. Aksfusarium og indhold af deoxynivalenol (DON) i forskellige hvedesorter under svampebehandling. DJF Rapport 66: 97-102 (2002).

Landsudvalget for Planteavl, 1998-2003. Oversigt over Landsforsøgene 1998, 1999, 2000, 2001, 2002 and 2003 (in Danish). Landbrugets Rådgivningscenter, Landskontoret for Planteavl. Skejby, Denmark.

Langseth W, Høie R, Gullord M. 1995. The influence of cultivars, location and climate on deoxynivalenol in Norwegian oats 1985-1990. *Acta Agric. Scand. Sect. B. Soil and Plant* 45: 63-67.

Langseth W, Kosiak B, Clasen P-E, Torp M, Gareis M. 1997. Toxicity and occurrence of *Fusarium* species and mycotoxins in late harvested and overwintered grain from Norway, 1993. *Journal of Phytopathology* 145: 409-416.

Langseth W, Stabbetorp H. 1996. The effect of lodging and time of harvest on deoxynivalenol contamination in barley and oats. *Journal of Phytopathology* 144: 241-245.

Langseth W, Elen O. 1997. The occurrence of deoxynivalenol in Norwegian cereals – differences between years and districts, 1988-1996. *Acta Agric. Scand. Sect. B. Soil and Plant Sci* 47: 176 – 184.

Miller JD. 1994. Epidemiology of *Fusarium* ear diseases of cereals. In: *Mycotoxins in Grain Compounds other than Aflatoxin*. Miller JD, Trenholm HL, editors. St. Paul: Eagan Press pp. 19 –36.

Muller H.-M, Reimann J, Schumacher U, Schwadorf K. 1997. *Fusarium* toxins in wheat harvested during six years in an area of Southwest Germany. *Natural Toxins* 5: 24-30.

1
2
3
4 Nusser SM., Carriquiry KW, Fuller WA. 1996. A semi-parametric transformation
5 approach to estimating usual intake daily intake distributions, Journal of the American
6 Statistical Association, 91, 1440-1449.

7
8
9 Pestka JJ, Smolinski AT, 2005. Deoxynivalenol: toxicology and potential effects on
10 humans. Journal of Toxicology and Environmental Health, Part B, 8: 39-69.

11
12
13 Pieters MN, Fiolet DCM, Baars AJ, 1999. Deoxynivalenol. Deviation of concentration
14 limits in wheat and wheat containing products. Bilthoven: National Institute of Public
15 Health and the Environment. Report no.: 388802018.

16
17
18 Placinta CM, D'Mello JPF, Macdonald AMC. 1999. A review of worldwide
19 contamination of cereal grains and animal feed with Fusarium toxins. Animal Feed
20 Science and Technology 78: 21-37.

21
22
23 Poulsen ME, Andersen JH., Petersen A, Hartkopp, H. 2005. Pesticides, Food
24 monitoring, 1998-2003. Part 2, Prepared by the Danish Institute for Food and
25 Veterinary Research for the Danish Veterinary and Food Administration, available:
26 http://www.dfvf.dk/Files/Filer/Pesticid/Monitoring_1998-2003.pdf .

27
28
29 Prelusky DB, Rotter BA, Rotter RG. 1994. Toxicology of mycotoxins. Mycotoxins in
30 grain: Compounds other than aflatoxin. Miller JD, Trenholm HL. St. Paul, Eagan Press
31 pp. 359-403.

32
33
34 Rasmussen PH, Ghorbani F, Berg T. 2003. Deoxynivalenol and other *Fusarium* toxins
35 in wheat and rye flours on the Danish market. Food Additives and Contaminants, 20:
36 396 – 404.

37
38
39 Razzazi-Fazeli E, Bohm J, Luf W. 1999. Determination of nivalenol and deoxynivalenol
40 in wheat using liquid chromatography-mass spectrometry with negative ion atmospheric
41 pressure chemical ionisation. Journal of Chromatography A, 854: 45-55.

42
43
44 Razzazi-Fazeli E, Rabus B, Cecon B, Bohm J. 2002. Simultaneous quantification of A-
45 trichothecene mycotoxins in grains using liquid chromatography-atmospheric pressure
46 chemical ionisation mass spectrometry. Journal of Chromatography A 968:129-142.

47
48
49 SCF. Opinion on *Fusarium* toxins. Part 1: Deoxynivalenol (DON), expressed on 2
50 December 1999. Scientific Committee on Food, 1999: SCF/CS/CNTM/MYC/19) Final.
51 Available at: <http://europa.eu.int/comm/food/fs/sc/scf/out44en.pdf>.

52
53
54 SCOOP, 2003, TASK 3.2.10: Collection of occurrence data of Fusarium toxins in food
55 and assessment of dietary intake by the population of EU Member States (available at:
56 <http://europa.eu.int/comm/food/fs/sc/scf/index-en.html>)

57
58
59 Scott PM. 1997. Multi year monitoring of Canadian grains and grain-based foods for
60 trichothecenes and zearalenone. Food Additives and Contaminants 14: 333-339.

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50
51
52
53
54
55
56
57
58
59
60

Sutton JC. 1982. Epidemiology of wheat head blight and maize ear rot caused by *Fusarium graminearum*. Canadian Journal of Plant Pathology. 4: 195 – 209.

Teich AH. 1989. Epidemiology of wheat (*Triticum aestivum* L.) scab caused by *Fusarium* spp, *Fusarium*, Mycotoxins, Taxonomy and Pathogenicity. Topics in Secondary Metabolites, vol. 2. Chelkowski J, editor. Amsterdam: Elsevier 492 pp.

Trucksess MW, Page SW, Wood GE, Cho T-H. 1998, Determination of deoxynivalenol in white flour, whole wheat flour, and bran by solid extraction/liquid chromatography: Interlaboratory study. J. AOAC International 81: 880-886.

Ueno, Y. 1983. Trichothecenes - Chemical, Biological and Toxicological Aspects. Developments in Food Science 4. Amsterdam: Elsevier 313 pp.

Widestrand J, Pettersson H. 2001. Effect of time, temperature and solvent on the stability of T-2 toxin, HT-2 toxin, deoxynivalenol and nivalenol calibrants. Food Additives and Contaminants 18: 407-414.

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Table I.

Occurrence of deoxynivalenol (DON) in flour samples of wheat collected during the survey period 1998 –2003.

Harvest year	DON ($\mu\text{g}/\text{kg}$)				Number of samples in each range ($\mu\text{g}/\text{kg}$)			
	Number of positives/total	Mean value ^a	Median value	Max. value	< 100	101-250	251-500	>500
1998	14/14	191	140	465	3	7	4	0
1999	16/16	175	167	527	4	8	3	1
2000	26/28	59	33	330	24	3	1	0
2001	19/30	32	10	204	27	3	0	0
2002	29/36	255	300	706	13	4	15	4
2003	26/27	148	151	224	6	21	0	0

a) Arithmetic mean. Values below the detection limit (20 $\mu\text{g}/\text{kg}$) are set to have a concentration of half the detection limit.

Table II

Mean values of precipitation and temperature for the whole country recorded each year between 1998 and 2003 for June, July and August. Precipitation and temperature data for the weeks 25, 26 and 27 from each year are also shown.

Year and week	Precipitation (mm)			Temperature (°C)		
	June (55)	July (66)	August (67)	June (14.3)	July (15.6)	August (15.7)
1998	76	92	60	13.9	14.4	14.6
<u>week 25, June 22-29</u>	<u>27</u>			<u>15.4</u>		
<u>week 26, June 30 – July 6</u>		<u>20</u>			<u>13.9</u>	
<u>week 27, July 7 - 13</u>		<u>25</u>			<u>13.4</u>	
1999	120	56	88	13.5	17.2	16.5
<u>week 25, June 22 – 28</u>	<u>22</u>			<u>12.7</u>		
<u>week 26, June 28 – July 5</u>		<u>18</u>			<u>15.7</u>	
<u>week 27, July 6 – 12</u>		<u>12</u>			<u>17.5</u>	
2000	53	42	48	13.7	14.9	15.2
<u>week 25, June 19- 26</u>	<u>21</u>			<u>17.3</u>		
<u>week 26, June 26 – July 3</u>	<u>8</u>			<u>12.6</u>		
<u>week 27, July 3 - 10</u>		<u>10</u>			<u>14.4</u>	
2001	62	47	91	12.8	17.5	17.0
<u>week 25, June 18-25</u>	<u>9</u>			<u>13.6</u>		
<u>week 26, June 26- July 2</u>	<u>12</u>			<u>16.6</u>		
<u>week 27, July 2-9</u>		<u>5</u>			<u>19.9</u>	
2002	100	113	71	15.6	17.1	19.6
<u>week 25, June 17-24</u>	<u>39</u>			<u>16.8</u>		
<u>week 26, June 24-July 1</u>	<u>24</u>			<u>13.9</u>		
<u>week 27, July 1-8</u>		<u>36</u>			<u>14.3</u>	
2003	80	72	41	15.8	18.1	17.7
<u>week 25, June 16-23</u>	<u>11</u>			<u>14.7</u>		
<u>week 26, June 23-30</u>	<u>23</u>			<u>16.3</u>		
<u>week 27, July 1-7</u>		<u>42</u>			<u>15.8</u>	

The mean values of the precipitation and temperature recorded each year between 1962 and 1995 are shown in parenthesis. Data were obtained from the Danish Meteorological Institute (DMI).

Table III. Background information for the deterministic calculation of the usual mean intake of DON from wheat and rye flour

	Total population		Children	
	Wheat	Rye	Wheat	Rye
Number of participants	4068	4068	807	807
Consumption days/days bread is consumed	28476/24269	28476/21513	<u>5649/4749</u>	<u>5649/4151</u>
Mean consumption of bread (g)	92	60	89	48
Estimated mean level of DON ($\mu\text{g}/\text{kg}$) in bread	109	26	109	26
Mean body weight	66.2 kg	66.2 kg	35.1 kg	35.1 kg
Mean intake in $\mu\text{g}/\text{kg}$ bw/day	0.15	0.02	0.28	0.04
Sum of intakes from wheat and rye bread	0.17 $\mu\text{g}/\text{kg}$ bw/day		0.32 $\mu\text{g}/\text{kg}$ bw/day	

Figure 1. Total ion chromatogram obtained after clean-up with Mycosep 225 column of a natural contaminated wheat extract containing 250 µg/kg DON. The mass spectrum in the negative ionisation mode (APCI/MS) is also shown.

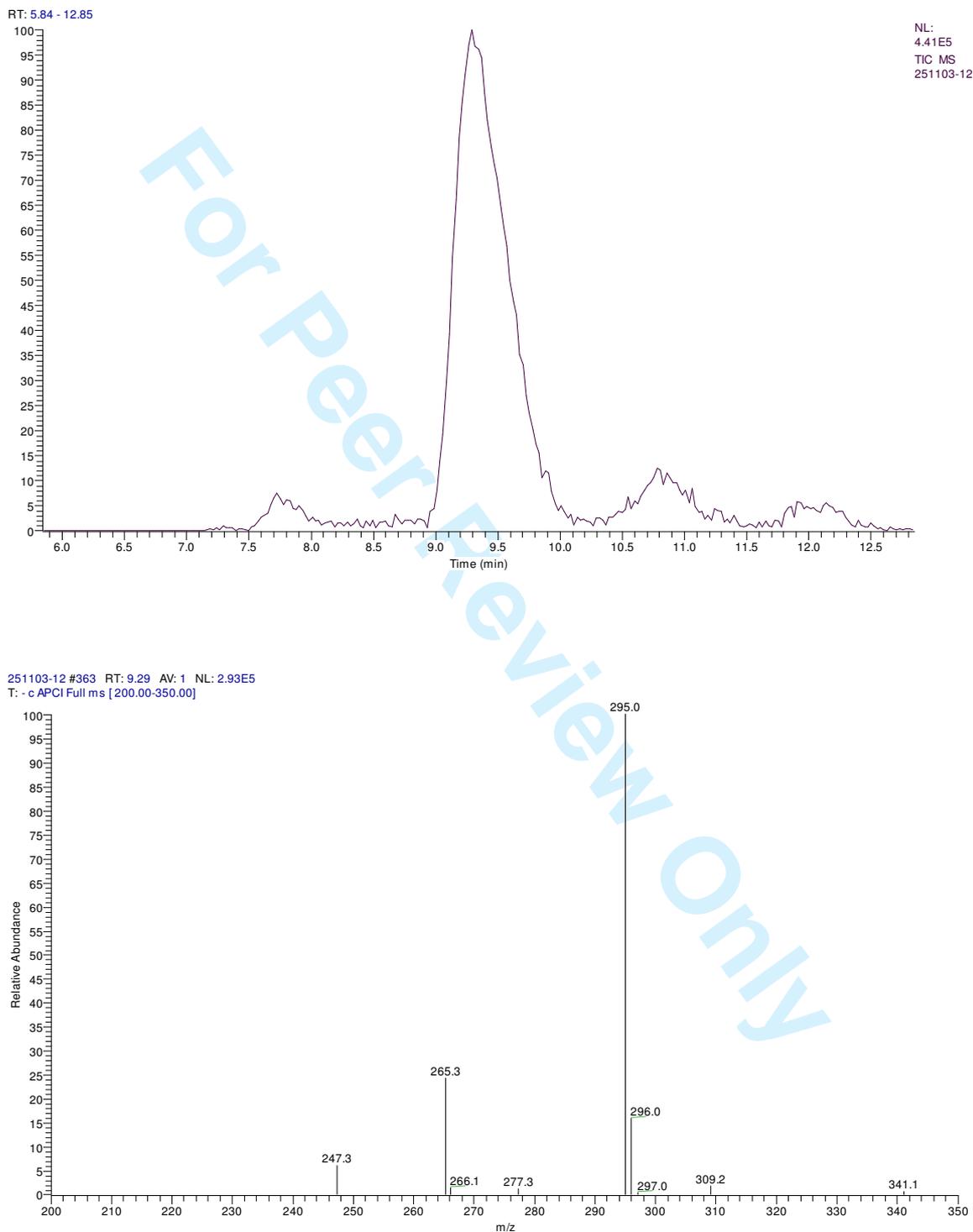


Figure 2. Correlation between DON concentrations measured in samples of common wheat flour and durum wheat flour by GC-ECD (x) and LC-MS (y). The linear regression line was $y = 1.08x - 28$ ($r^2 = 0.983$)

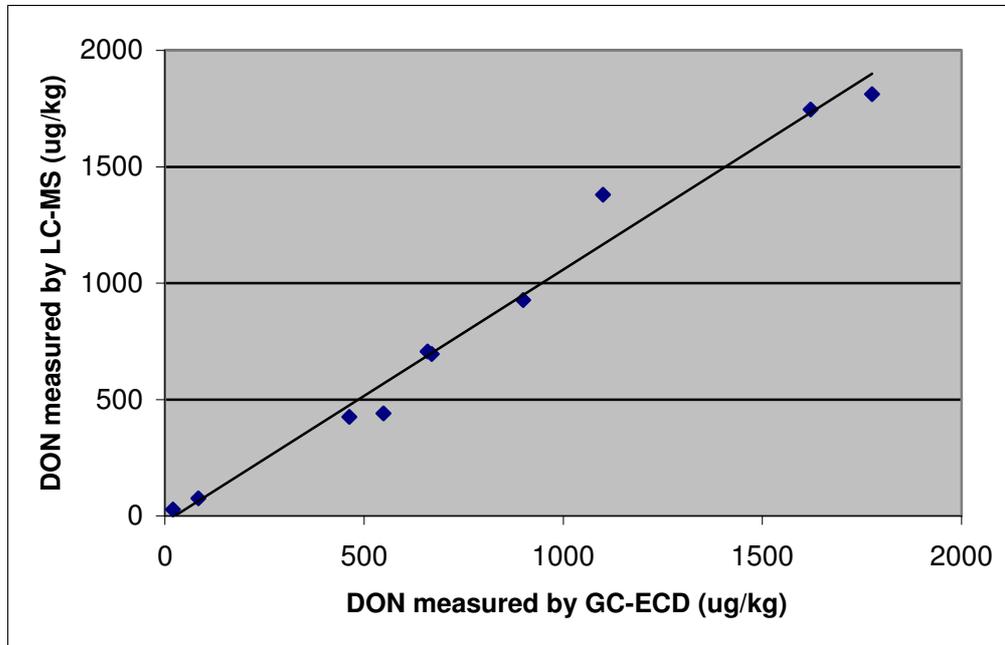


Figure 3. Mean intake and percentiles for the intake of DON for children (dark column) and the total Danish population (light column) determined by the probabilistic method.

