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Simultaneous determination of type A, B, and D trichothecenes and their occurrence in cereals and cereal products

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Running title: Determination of type A, B and D trichothecenes by LC-MS/MS

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Abbreviations: 3-acDON, 3-acetyl-deoxynivalenol; 15-acDON, 15-acetyl-deoxynivalenol; C13-DON, $^{13}C_{15}$-deoxynivalenol; C13-T-2, $^{13}C_{24}$-T-2 toxin; DAS, 4,15-diacetoxyscirpenol; DacVOL, 4,15-diacetylverrucarol; DON, deoxynivalenol; EC, European Commission; FX, fusarenon-X; LOD, limit of detection; LOQ, limit of quantification; MAS, 15-monoacetoxyscirpenol; NEO, neosolaniol; NIV, nivalenol; RA, roridin A; SG, satratoxin G; SH, satratoxin H; TDI, tolerable daily intake; VA, verrucarin A; VOL, verrucarol

Abstract

A sensitive LC-MS/MS method for the simultaneous determination of type A, B, and D trichothecenes in cereals is presented. The limits of detection ranged between 0.1 µg kg^{-1} and 0.7 µg kg^{-1} for all analytes. The method was applied to 289 representatively drawn samples of wheat, rye, and oat products. Ninety-four percent of the wheat samples (n=130), 95% of the rye samples (n=61), and 100% of the oat samples (n=98) were contaminated with the type A trichothecenes T-2 and HT-2 toxin. Median levels of T-2/HT-2 (sum of the toxins) were 0.91 µg kg^{-1}, 0.53 µg kg^{-1}, and 8.2 µg kg^{-1}, respectively. Highest levels were found in wheat bran (24 µg kg^{-1}), rye kernels (3.1 µg kg^{-1}), and oat flakes (85 µg kg^{-1}). All wheat and rye samples and 75% of the oat samples were contaminated with the type B trichothecene deoxynivalenol. Median levels of this toxin were 23 µg kg^{-1}, 15 µg kg^{-1}, and 0.53 µg kg^{-1}, respectively. Highest levels were found in wheat bran (1160 µg kg^{-1}), rye kernels (288 µg kg^{-1}), and oat flakes (55 µg kg^{-1}). The type B trichothecene nivalenol was detected in 67% of the wheat samples, in 3% of the rye samples, and in 24% of the oat samples with highest levels in wheat bran (96 µg kg^{-1}), rye kernels (1.8 µg kg^{-1}), and in oat flakes (17 µg kg^{-1}), respectively. Levels of other type A and B trichothecenes played a minor role, although the rates of contamination were often high. Neither macrocyclic type D trichothecenes (satratoxin G and H, verrucarin A, roridin A) nor the type A trichothecenes diacetylverrucarol and verrucarol were detected in any of the samples.

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Introduction

Three to four hundered secondary fungal metabolites have been recognized as mycotoxins (Bennett et al. 2003). Of these almost 150 belong to the large group of trichothecenes. These are cyclic sesquiterpenoids characterized by a 12,13-epoxide ring (WHO 1990). Trichothecenes are generally known as cytotoxic and immunosuppressive mycotoxins. They are mainly produced by certain species of *Fusarium*, as well as by species of the genera *Myrothecium*, *Stachybotrys*, *Trichothecium,* and *Trichoderma*. Trichothecenes are divided into four groups according to their chemical structures (Ueno 1983a). Type A trichothecenes are characterized by an oxygen function different from a carbonyl group at the C-8 position while type B trichothecenes possess a carbonyl function at this position. Type C trichothecenes have a second epoxide group at C-7,8 or C-9,10 whereas type D trichothecenes contain a macrocyclic ring between C-4 and C-15 (Figure 1). *F. sporotrichioides* and *F. langsethiae* are the main producers of type A trichothecenes like T-2 and HT-2 toxin in European cereals (Torp et al. 2004, Thrane et al. 2004). Oats and maize are considered to be most contaminated with this group of trichothecenes. Type B trichothecenes like deoxynivalenol (DON) are primarily produced by *F. graminearum* and *F. culmorum* which play a major role in maize, wheat and rye (SCOOP 2003). Various multi-methods for the determination of these toxins in cereals have been developed in the past few years (Lagana et al. 2003, Berthiller et al. 2005, Biselli et al. 2005, Klötzel et al. 2005). The macrocyclic type D trichothecenes are metabolites of *Myrothecium* spp. (e.g. roridin A, verrucarin A) and *Stachybotrys* spp. (e.g. satratoxin G and H; Ueno 1983a). While type A and B trichothecenes are commonly known as contaminants of food and feed, it is assumed that macrocyclic trichothecenes rarely occur in these matrices (WHO 1990, Krska et al. 2001). However, these toxins have occasionally been determined in hay or straw and were identified as causative agents of stachybotryotoxicosis (Jarvis et al. 1986), a severe disease that primarily affects horses. During the last years, macrocyclic trichothecenes have attracted more attention as indoor pollutants. They are suspected to be responsible for several adverse health effects in humans living or working in *Stachybotrys*-affected environments (Johanning et al. 1996, Jarvis et al. 1998, Gottschalk et al. 2008). Regarding food, there are only a few studies

focussing on these analytes. In 1980, Stack and Eppley first described a method for cereal grains

with LODs between 100 and 200 µg kg^{-1}, but no naturally contaminated samples were analyzed

(Stack and Eppley 1980). Recently published and more sensitive methods for type A, B and D

trichothecenes in foodstuffs (Gentili et al. 2007, Stecher et al. 2007) comprised no satratoxins.

Furthermore, only a very small number of samples was analyzed.

Maximum levels for trichothecenes in cereals and corn currently only exist for DON

(Commission Regulation (EC) No 1881/2006 and No 1126/2007). According to mean intake

data provided by a study from the Scientific Cooperation, the dietary exposure to T-2 and HT-2

may be critical. Especially in the group of infants and children the temporary tolerable daily

intake (tTDI) for the sum of these toxins could be exceeded by more than fivefold (SCOOP

2003). The EC thus requested more occurrence data of these toxins in order to establish an

appropriate maximum level (Commission Regulation (EC) No 1881/2006).

A sensitive LC-MS/MS multi-trichothecene method was developed in this study. Data regarding

the contamination rates and levels of type A, B and the (at least in food) hardly studied type D

trichothecenes in representatively drawn wheat, rye and oat samples are presented. Furthermore,

the levels of mycotoxin contamination are compared and evaluated with respect to the possible

health risks for the consumer.

Materials and methods

Samples

Sampling was performed according to Commission Regulation (EC) No 401/2006. Samples

(total n=289) of wheat products (n=130), rye products (n=61), and oat products (n=98) were

collected from grain milling factories or from wholesale in Bavaria. Products under study

included kernels, flour, semolina, bran, and flakes. A small number of these samples (n=18)

were oat- (n=13) or wheat-containing (n=5) infant food. All samples were of German origin

from the crop years 2005 and 2006. The grain kernel samples were fully processed (including

cleaning and dehulling steps), suitable for direct consumption. In addition to these food grain

samples, 17 samples of oats of feed quality were included in this study.

Chemicals and reagents

Standards of the type A trichothecenes T-2 toxin, HT-2 toxin, 4,15-diacetoxyscirpenol, 15-

monoacetoxyscirpenol, T-2 triol, T-2 tetraol, neosolaniol, 4,15-diacetylverrucarol, and

verrucarol, standards of the type B trichothecenes deoxynivalenol, nivalenol, fusarenon-X, 3-

and 15-acetyldeoxynivalenol as well as standards of the type D trichothecenes roridin A and

verrucarin A were purchased from Sigma (Deisenhofen, Germany). 13C-stable isotope

standards for DON and T-2 toxin were from Biopure (Coring System Diagnostix, Gernsheim,

Germany). Acetonitrile for extraction (HPLC grade) was purchased from Riedel-de Haën

(Deisenhofen, Germany). Methanol (Riedel-de Haën, Deisenhofen, Germany) and ammonium

formate (Fluka, Deisenhofen, Germany) were used in LC-MS quality. Water was purified on a

milli-Q water purification system (Millipore, Schwalbach, Germany).

Standards of the type D trichothecenes satratoxin G and H were produced in our laboratory. A

toxigenic strain of *Stachybotrys chartarum* was cultivated on rice (Jarvis et al. 1986). After a

period of eight weeks at room temperature, cultures were extracted with acetonitrile/water 84/16

(v/v) and the extracts were cleaned using MycoSep® #226 columns. The purified extracts were

fractionated by semi-preparative HPLC with MS/MS-detection using a T-fitting to collect the

fractions. A Beckman ultrasphere column (ODS, 150×4.6 mm, 5 µm) and a binary linear

gradient of methanol (pump A) and deionized water (pump B) was used for separation of the

toxins at a flow rate of 1000 µl/min: 0 min 90% B, 40 min 0% B, 41 min 90% B, 45 min 90%

B. The injection volume was 200 µl. The fractions were evaporated to dryness using a gentle

stream of nitrogen at 40 °C. The crystalline residues were then dissolved and stored in

acetonitrile. The standards were verified and quantified using reference substances which were

kindly provided by Manfred Gareis (Max-Rubner Institute, Kulmbach, Germany).

Sample preparation

The complete aggregate samples (between 1 and 10 kg) were finely ground and homogenized before analysis. The sub-samples (20 g) were extracted with 80 ml acetonitrile/water 84/16 (v/v) by blending at high speed (10000 rpm, 3 min) with an Ultra Turrax homogenizer (IKA-Labortechnik, Staufen, Germany). For some infant food samples the double volume of extraction solvent was used because of their high swelling properties. The extracts were centrifuged (6000g, 10 min) and aliquots of 8 ml were purified with MycoSep® columns (# 226, Coring System Diagnostix, Germany) according to the manufacturer's instructions. The MycoSep® method has successfully been applied by many other working groups (Berthiller et al. 2005, Biselli et al. 2005, Klötzel et al. 2005). As also observed by Stecher et al. (2007), the commonly used MycoSep® trichothecene column #227 was not suited for type D trichothecenes. Their high charcoal content led to very low recoveries (data not shown). 4 ml of the extracts were evaporated to dryness with a gentle stream of nitrogen at a temperature of 40 °C (Barkey, Leopoldshöhe, Germany). For LC-MS/MS analysis the residues were dissolved in 1 ml of methanol/deionized water 5/95 (v/v) and were filtered through a 0.45 µm PTFE syringe filter (Supelco, Deisenhofen, Germany).

LC-MS/MS instrumentation and parameters

LC-MS/MS analysis was performed with a high pressure gradient HPLC apparatus from Shimadzu (Duisburg, Germany) and a triple quadrupole mass spectrometer API 4000 from Applied Biosystems (Darmstadt, Germany). The LC system consisted of an autosampler (SIL HT-C), two pumps (LC-10ADVP), a degasser (DGU-14A), and a column oven (CTO-10ACVP). A Synergi™ polar-RP® column, 150 x 2 mm, 4 µm (Phenomenex, Aschaffenburg, Germany) was used for the chromatographic separation of the analytes. The binary linear gradient consisted of eluent A (methanol + 5 mmol/l ammonium formate) and eluent B (deionized water) with a flow rate of 0.4 ml/min: 0 min 95% B, 11 min 95% B, 22 min 35% B, 26 min 35% B, 27 min 95% B, 35 min 95% B. It was not possible to analyze T-2 tetraol and NIV in a single run due to similar retention times and ionization with different polarities. T-2

tetraol was determined in a second run with the following linear gradient: 0 min 70% B, 3 min 70% B, 5 min 30% B, 11 min 30% B, 11.5 min 70% B, 13 min 70% B. The column oven temperature was maintained at 40 °C and the injection volume was 20 µl.

MS experiments were carried out in positive and negative electrospray ionization (ESI) mode by separating the method in periods with the proper polarity (Figure 2). The source temperature was set at 500 °C and the nitrogen pressure of the nebulizer gas (GS1) was optimized at 55 psi. The collision gas (CAD-gas, nitrogen) was used in high mode (CAD=9). The pressure of the curtain gas for T-2 tetraol was set at 25 psi, the heating gas at 55 psi, and the ion spray voltage at 3000 V. For the other toxins best sensitivity was achieved with 20 psi, 50 psi and 4500 V, respectively. In positive ionization mode, all analytes were measured as adduct ions of ammonium $[M+NH_4]^+$. The toxins were identified in multiple reaction monitoring mode (MRM) by two specific fragment ions of one precursor ion according to Commission Decision 2002/657/EC. The MRM-transitions, de-clustering potentials (DP), collision energies (CE), cell exit potentials (CXP), and further parameters for each toxin are shown in Table 1. The value of the entrance potential (EP) was +10 V or -10 V for all analytes depending on their ionization polarity.

Method validation

The method performance parameters were determined according to DIN 32645 (1994). The trichothecene stock solution was diluted into 25 concentrations between 250 ng ml^{-1} and 0.005 ng ml^{-1}. These standard dilutions were measured to determine the linearity of the method, the correlation as well as the limits of detection (LODs) and of quantification (LOQs). The latter were also calculated using the signal-to-noise (S/N) approach (S/N=3 and S/N=9, respectively). The repeatability of the method was checked by five-fold measurements of the toxin standards at two concentrations (1.0 and 10 ng ml^{-1}).

Recovery experiments were carried out after artificial contamination of homogenized, blank samples of wheat flour, rye flour, and ground oat flakes on three levels (1.0 µg kg^{-1}, 10 µg kg^{-1}, and 50 µg kg^{-1}) with five replicates each. The samples were fortified with a trichothecene standard mixture before extraction.

The accuracy of the method was checked by participation at an interlaboratory test for DON (DLA, Ahrensburg, Germany) and intra laboratory comparisons between the results of LC-MS/MS and HPLC-FLD (post-column derivatization) measurements according to the method described by Engelhardt et al. (2006). Comparison measurements for T-2 and HT-2 in oat flakes were conducted with another laboratory.

Mycotoxin quantification

Quantification of most of the toxins was done by external calibration on five levels (0, 1.0, 10, 50, 200 ng ml^{-1}). The ^{13}C-stable isotope standards ^{13}C-DON and ^{13}C-T-2 toxin were used for compensation of matrix suppression effects, which mainly occurred in wheat samples. After evaporation of the purified extracts, the residues were dissolved in eluent containing the ^{13}C-standards at a concentration of 10 ng ml^{-1}. To compensate for matrix effects which were also observed for NIV and T-2 tetraol, these two toxins were quantified by matrix assisted calibration. Therefore, extracts of blank wheat flour, rye flour, and ground oat flakes were used for the dilution of the toxin standards. Levels of toxins between the LOD and LOQ were calculated with half LOQ, at which the S/N-values were applied. All results were corrected by mean recovery rates for each toxin and matrix referring to the results of the recovery experiments.

Results

Method performance characteristics

According to DIN 32645 LODs/LOQs between 0.09/0.29 µg kg^{-1} for DAS and 0.70/2.2 µg kg^{-1} for NIV were calculated. Using the signal-to-noise approach, these limits were mostly lower

(Table 2). The repeatability (n=5) as RSD was between 1.8% and 11% at a concentration of 1 ng ml^{-1}. At a level of 10 ng ml^{-1}, all RSDs were between 0.5% and 4.4%. The method was linear between the LODs and 250 µg kg^{-1} except for HT-2, VOL, and SH (LOD - 200 µg kg^{-1}) with correlation coefficients (linear regression) of r > 0.9980 (Table 2).

The average recovery obtained with this method ranged between 60 and 108% for all toxins and matrices with RSDs between 1.3% and 17%. The lowest recoveries were observed for NIV and T-2 tetraol (Table 3). The accuracy of the method was confirmed by participation in an interlaboratory test for DON and comparison measurements for DON and T-2/HT-2. The results showed a satisfactory accordance (Table 4). Finally, the results of the validation measurements were considered to comply with the requirements of the EC (Commission Regulation (EC) No 401/2006).

Trichothecene contamination of wheat samples

The samples (n=130) were analyzed for 18 type A, B and D trichothecenes. The levels of T-2/HT-2 in all wheat samples ranged between LOD and 24 µg kg^{-1} with a contamination rate of 94% and a median contamination of 0.91 µg kg^{-1}. The 95th percentile value was 6.4 µg kg^{-1} (Table 5). Hereby, a correlation between T-2 and HT-2 levels was observed (R^2=0.7995). The levels of HT-2 were about sevenfold as high as those of T-2 (Figure 3). As compared in Figure 4, the highest levels were found in wheat bran. Levels of contamination of the other commodities of wheat are also listed in Table 5. T-2 tetraol occurred in 53% of all samples with values between LOD and 83 µg kg^{-1} in a wheat bran sample. The median level, however, was only 0.15 µg kg^{-1}. Other type A trichothecenes like T-2 triol, NEO and DAS were detected with lower frequency and in smaller amounts (Table 5). The rate of contamination with MAS was 92% but the levels were consistently low (median 0.11 µg kg^{-1}, maximum value 5.9 µg kg^{-1} in wheat bran). VOL and DacVOL were not detected above their LODs.

All wheat samples were contaminated with DON between 0.06 and 1160 µg kg^{-1}. The median contamination was 23 µg kg^{-1} with a 95th percentile value of 162 µg kg^{-1} (Table 5). Again, the highest value was found in bran (Figure 4). However, this was the only sample to exceed the maximum level set by the EC (Commission Regulation No 1881/2006). Sixty-seven percent of the samples were contaminated with NIV at a median level of 1.8 µg kg^{-1} and a maximum level of 96 µg kg^{-1} in wheat bran. The 3- and 15-acetyl metabolites of DON were detected in 52 and 72% of all wheat samples with median values of only 0.21 µg kg^{-1} and 0.17 µg kg^{-1}, respectively. The highest levels of 15 µg kg^{-1} and 26 µg kg^{-1} were also found in wheat bran (Table 5). 15-acDON-levels were about 2% of the DON-levels (Figure 3). No type D trichothecenes were detected above their LODs between 0.02 µg kg^{-1} for RA and 0.15 µg kg^{-1} for SH.

Trichothecene contamination of rye samples

Rye samples (n=61) were less contaminated with trichothecenes, even though the rates of contamination hardly differed from those of wheat. The median level of T-2/HT-2 in all rye samples was 0.53 µg kg^{-1} with a the 95th percentile value of 1.8 µg kg^{-1}. The rate of contamination was 95% (Table 6) and a maximum level of 3.1 µg kg^{-1} was measured in rye flour (Figure 4). Higher levels (median 2.0 µg kg^{-1} and 95th percentile value 6.4 µg kg^{-1}) were observed for T-2 tetraol with a rate of contamination of 79% and a maximum level of 8.1 µg kg^{-1} in rye kernels (Table 6). DAS, VOL and DacVOL were not detected and the levels of MAS and NEO were very low. Due to the low T-2 and HT-2 levels, the correlation between these two toxins was less evident than in wheat or oats (Figure 3).

However, all samples were contaminated with DON. The median contamination with this toxin was 15 µg kg^{-1} and the 95th percentile value was 92 µg kg^{-1}. The highest value of 288 µg kg^{-1} was found in rye kernels (Table 6). 3-acDON and 15-acDON were detected with mean levels of lower than 1 µg kg^{-1} in 59% and 80% of the samples. It could be observed that the amounts of

DON were about 30 times as high as the levels of 15-acDON (Figure 3). NIV and FX levels were almost all below the LOD. No type D trichothecenes were detected.

Trichothecene contamination of oat samples

All oat samples (n=98) were contaminated with T-2/HT-2 between 0.04 and 85 µg kg^{-1} (Table 7). The median and 95th percentile value were 8.2 µg kg^{-1} and 50 µg kg^{-1}. The highest levels were measured in fine oat flakes (Figure 4). All five samples (5.1%) which exceeded a level of 50 µg kg^{-1} for the sum of T-2/HT-2 were part of this commodity. The T-2 levels in oats were about half the levels of HT-2 (Figure 3). Within the group of type A trichothecenes, T-2 tetraol also played a major role (rate of contamination 90%). The median level was 6.4 µg kg^{-1} (95th percentile value: 25 µg kg^{-1}).. The highest value of 85 µg kg^{-1} was measured in a baby food sample (Table 7). For T-2 triol, NEO, DAS, and MAS, levels of at most 2.7 µg kg^{-1} were determined. VOL and DacVOL were not detected in any sample.

The contamination rate of DON was 74% of all oat samples. The median value was 0.53 µg kg^{-1} and the 95th percentile 11 µg kg^{-1}. A maximum value of 55 µg kg^{-1} was determined in fine oat flakes (Table 7). DON-levels of different commodities of oats are compared in Figure 4. 3-acDON and 15-acDON were measured each with a rate of contamination of 28% in all oat samples and with maximum levels of 8.2 µg kg^{-1} and 1.4 µg kg^{-1}. These levels were too low to observe a good correlation with DON (Figure 3). NIV occurred in 24% of the oat samples with a maximum level of 17 µg kg^{-1} found in fine oat flakes (Table 7). The highest contamination with FX (6.1% positive samples) was below 1.0 µg kg^{-1}. Again, no type D trichothecenes were detected.

Trichothecene contamination of infant food

In 100% of the oat-containing baby food samples (n=13) and in 80 % of the wheat-containing samples (n=5) T-2/HT-2 was detected. The median values were 6.0 µg kg^{-1} and 0.36 µg kg^{-1}, respectively (Figure 4). The highest value of 23 µg kg^{-1} was found in an oat porridge powder

which was concurrently contaminated with 85 µg kg^{-1} T-2 tetraol (Table 7). Otherwise, the levels of T-2 tetraol were low just like those of the other analytes. In Figure 4, also the DON levels of oat and wheat-containing infant food were compared to the other food commodities. DON was detected in 54% and 100 % of these samples with median values of 0.17 µg kg^{-1} and 9.9 µg kg^{-1} (Table 5). The highest level of 24 µg kg^{-1} was detected in a semolina powder (Table 5). 3-acDON and 15-acDON were detected with levels below 3.2 µg kg^{-1}. Three samples contained NIV with maximal 8.8 µg kg^{-1} (Table 5) and only one sample FX (0.18 µg kg^{-1}, Table 7).

Trichothecene contamination of feed oats

Oats of feed quality (n=17) were contaminated at a much higher level with type A and B trichothecenes due to the fact that the samples were husked oat kernels. The toxin levels are usually reduced about 90% by de-husking the grains (Scudamore et al. 2007). Maximum values of 531 µg kg^{-1} T-2/HT-2 and 654 µg kg^{-1} T-2 tetraol in the same sample were measured. The median/95th percentile values were 162 µg kg^{-1}/356 µg kg^{-1} and 146 µg kg^{-1}/331 µg kg^{-1}, respectively (rates of contamination 100%). T-2 tetraol again contributed considerably to the total type A trichothecene burden. All samples contained DAS (median/95th percentile value: 0.61 µg kg^{-1}/3.2 µg kg^{-1}), MAS (1.6 µg kg^{-1}/6.3 µg kg^{-1}), and NEO (4.7 µg kg^{-1}/17 µg kg^{-1}). For NEO, comparatively high values up to 28 µg kg^{-1} were determined. Thereby, a strong correlation was also observed between the levels of T-2 and NEO. In this matrix, the levels were high enough to not be influenced by the LOQ of the method. The NEO-level was about 15% of the one of T-2 (R^2=0.9496). No VOL or DacVOL were measured.

All samples were concurrently contaminated with DON (median/95th percentile value: 58 µg kg^{-1}/678 µg kg^{-1}) and NIV (57 µg kg^{-1}/189 µg kg^{-1}). Two samples had exceptionally high levels of 2400 µg kg^{-1} and 490 µg kg^{-1}, respectively. The contamination rates of 3-acDON, 15-acDON, and FX were 53%, 24%, and 29%, respectively. The levels of 3-acDON (median/95th percentile value: 0.76 µg kg^{-1}/32 µg kg^{-1}), 15-acDON (<LOD/3.8 µg kg^{-1}), and FX (<LOD/3.4 µg kg^{-1}) were still low, although they remarkably differed from those of the food samples. Only the

sample with the highest DON-contamination contained a mentionable amount of 99 µg kg^{-1} of 3-acDON. However, no type D trichothecenes were detected.

Discussion

Type A trichothecenes

Oats and oat products had the highest levels of contamination with type A trichothecenes (Table 7). However, the comparatively low contamination of wheat should not be disregarded because of its much higher intake. In SCOOP task 3.2.10 (2003), wheat was identified as the predominant source of T-2/HT-2, but also of DON and NIV intake. As reported in the SCOOP study, oat samples were most frequently contaminated with HT-2 (rate of contamination 41%), followed by maize (24 %), rye (17%), and wheat (12%). The highest contamination rate with T-2 (28%) was found in maize (oats 16%, wheat and rye 21%, each). In this project, however, 100% of the oat samples, 94% of the wheat samples and 95% of the rye samples contained T-2/HT-2, which can be traced back to the much lower detection limits of this method. Thus, also the rates of contamination of T-2 triol, DAS, MAS, and NEO were much higher (Tables 5-7) in this study. However, the concentrations of these analytes were all below the LODs of the methods applied in the SCOOP study (2003), where only 1% to 6% of the samples were positive for these analytes. The LODs for type A trichothecenes in wheat, rye or oats were between 2 and 20 µg kg^{-1} in dependence of the analyte and the contributing laboratory. Like in this study, VOL was not detected, but no samples were analyzed for T-2 tetraol or DacVOL. However, data on the occurrence of T-2 tetraol were provided by Klötzel et al. (2005) and Schollenberger et al. (2006). Both reported high levels of this analyte in oats which were even higher than T-2 or HT-2, also supporting our results.

In comparison to the concentrations of type A trichothecenes found in this study, the levels reported in SCOOP (2003) were much higher. T-2 levels of wheat were between 2 and 160 µg kg^{-1}, HT-2 levels between 3 and 50 µg kg^{-1}. In rye, T-2 values ranged between 10 and 190 µg kg^{-1}. HT-2 values of between 10 and 70 µg kg^{-1} were determined. T-2 levels in oats ranged

between 10 and 550 µg kg^{-1} and HT-2 levels between 10 and 1150 µg kg^{-1} (SCOOP 2003). The

values of the few T-2 triol, DAS, MAS, and NEO-positive samples were between 30 and 50 µg

kg^{-1}. These higher levels may be a result of differing origins or crop years. It also has to be

considered that the samples analyzed here were all at least cleaned and de-hulled cereals

intended for human consumption. It has been shown that raw cereal kernels contain a tenfold

higher toxin amount than de-hulled kernels (Scudamore et al. 2007). Furthermore, Northern

European countries contributed results to the SCOOP study where usually the highest T-2/HT-2

levels are determined (Langseth et al. 1999, Pettersson et al. 2006).

The correlations between T-2 and HT-2 in the different cereal types gave interesting results.

While in wheat the level of HT-2 was about sevenfold as high as the T-2 level, HT-2 levels in

oats were only about twice as high as those of T-2. The contamination levels of rye were

however too low to be able to make proper comparisons (Figure 3). These different levels may

be due to the ability of the plant to metabolize T-2 to HT-2 which has been described for species

of *Baccharis* (Mirocha et al. 1988). The synthesis of deacetylases may be plant-specific. In this

context, also deacetylases originating from bacteria of the plant can play a role in metabolizing

processes (Beeton et al. 1989, Ueno et al. 1983b). Differences in the correlations between T-2

and HT-2 levels may be due to differing bacterial flora of each plant. However, it is

questionable whether these processes could lead to such considerable correlations. It seems

more likely that the different cereal types are infected by different *Fusarium* species or strains

which possess characteristic metabolite production abilities.

Anyway, oat products had the highest levels of contamination with T-2/HT-2 as well as T-2

tetraol. It should be noticed that the predominantly consumed oat products, fine oat flakes, were

the food commodity with the highest levels (Figure 4). Especially infants and children have a

high intake of these products by the consumption of muesli (see also "dietary intake

estimation"). This should be of concern to official food control authorities and for future

legislation.

Type B trichothecenes

All wheat and rye samples were contaminated with DON, while 74% of the oat samples contained this analyte. The mean contamination level of rye (28 µg kg^{-1}) was about half the level of wheat (57 µg kg^{-1}) while the mean DON-burden of oats was comparably low (2.8 µg kg^{-1}; Tables 5-7). In the SCOOP study (2003), DON was detected in only 61% of the wheat samples (mean level of all contributing countries: 205 µg kg^{-1}), in 41% of the rye samples (42 µg kg^{-1}), and in 33% of the oat samples (95 µg kg^{-1}). The rates of contamination as well as the toxin levels were influenced by the LODs of the different methods applied in the SCOOP study (2.0 to 220 µg kg^{-1}). In wheat the levels of DON ranged between 2.0 and 50000 µg kg^{-1}, between 2.0 and 595 µg kg^{-1} in rye, and between 2.0 and 5004 µg kg^{-1} in oats. Engelhardt et al. (2006) reported mean DON levels in wheat of 138 µg kg^{-1}. Mean levels in wheat and oats reported by Biselli et al. (2005) were 230 and 250 µg kg^{-1}. Similar results were shown by Schollenberger et al. (2002). The levels found in this study were much lower, which may be a result of the same reasons discussed above (Scudamore et al. 2007).

In the SCOOP study (2003), only 8.0% of the wheat samples, less than 1.0% of the rye samples and 6.0% of the oat samples contained 3-acDON, but at least 20% of all samples 15-acDON. Much higher rates of contamination were determined in this study (Tables 5-7). It was shown that the levels of the acetyl metabolites of DON were only about 2 to 3% as high as the DON-levels of wheat and rye. Due to the low DON-levels in oats, no correlation could be observed in this matrix (Figure 3). Basically, the 15-acDON levels were higher than those of 3-acDON. This resulted in more positive samples which exceeded the LOD. These results are in accordance with Klötzel et al. (2005).

For NIV, rates of contamination of 14% of wheat (values between 2.0 and 440 µg kg^{-1}), 5.0% of rye (values between 2.0 and 48 µg kg^{-1}), and 21% of oats (values between 2.0 and 1860 µg kg^{-1}) were reported in SCOOP data (2003). In spite of the more sensitive method applied here, the

contamination rates of rye and oats from SCOOP data were similar to our results (Tables 5-7).

However, 67% of the wheat samples were found to be NIV-positive, but with much lower

contamination levels as a result of the above mentioned reasons.

The rates and levels of contamination with FX were extremely low in this study (Tables 5-7).

Schollenberger et al. (1999, 2002) and Klötzel et al. (2005) also reported no positive cereal-

based food samples, wheat or oat samples (LODs: 6 µg kg^{-1} and 1.3 µg kg^{-1}). However, 10% of

the wheat samples from SCOOP contained FX between 3.3 µg kg^{-1} and 50 µg kg^{-1}.

Type D trichothecenes

To our knowledge, this was the first measurement of type D trichothecenes in a multitude of

representative cereal food samples (n=289) since 1980, when a much less sensitive method was

applied (Stack and Eppley 1980). Thus it was necessary to develop a sensitive LC-MS/MS

method to provide more up-to-date occurrence data. No samples contained satratoxin G or H,

roridin A or verrucarin A above their LODs. Actually, it can be concluded that *Stachybotrys*

spp. and *Myrothecium* spp. metabolites did not represent a risk to food security, at least as

regarding wheat, rye and oat products. The number of horse feed samples (raw oats, n=17) was

too low to draw proper conclusions, but no sample contained macrocyclic trichothecenes.

However, results of Kluwe revealed that stachybotryotoxicosis still is a risk in horse feeding. At

least 20% of 100 hay and straw samples contained *Stachybotrys* spp. Altogether, 32 strains have

been isolated and were tested for their toxinogenicity. More than 35% of these strains possessed

the tri5-gene and actually produced satratoxin G and H as examined by LC-MS/MS (Kluwe

2006).

Estimation of dietary intake

The toxicity of DON, NIV, and T-2/HT-2 was evaluated by the Scientific Committee on Food

(SCF) in a set of opinions (SCF 1999-2002). In a group evaluation of these four analytes, the

Committee concluded that combined or synergistic effects were not probable, although currently

available data on this matter was limited. Hence, the Committee confirmed the single TDI-values or temporary TDI-values (t-TDI) for each toxin as follows:

- DON: TDI = 1 µg kg^{-1} body weight (bw)/day

- NIV: t-TDI = 0.7 µg kg^{-1} bw/day

- T-2/HT-2: t-TDI = 0.06 µg kg^{-1} bw/day

In the following, intake estimations for DON, NIV and T-2/HT-2 were conducted for young children and infants. These are the consumer groups with the highest risk, as they have an exceptionally high intake in relation to their body weight. Hereby, data on the average consumption of wheat flour, rye flour, oat flakes, and oat-containing baby food were used (Table 8). The average body weight of nine-month-old infants and two- to five-year-old children were quoted with 8.95 kg and 16.15 kg (Kersting et al. 1998, Banasiak et al. 2005). The calculations were conducted for a mean case scenario (average consumption × median toxin concentration) and a bad case scenario (average consumption × 95th percentile value of toxin concentration). The corresponding levels of contamination can be gleaned from Tables 5-7.

The daily toxin intake by average consumption of selected food commodities and the percentage of the corresponding TDI-values is shown in Table 8. In the SCOOP study (2003) wheat was identified as the major source of T-2/HT-2 as well as DON and NIV intake. As expected, this was re-confirmed at least with regard to DON and NIV. However, the consumption of oat flakes was shown to play a major role in T-2/HT-2 exposure in this study. The contribution of rye, however, seemed to be negligible. Assuming the mean case scenario, the total T-2/HT-2 intake by the daily ingestion of wheat and rye flour as well as oat flakes barely corresponded to 10% of the t-TDI, whereas the intake by baby food was more than 30% of the t-TDI (Table 8). It must be pointed out that the number of baby food samples examined in this study was too small to justify proper conclusions. However, the T-2/HT-2 levels of oat-containing baby food (n=13) indicated a possible exceedance of the t-TDI in the sub-population of infants giving cause for

further study. Furthermore, it should be mentioned that not only T-2/HT-2 primarily accounted for the total type A trichothecene burden but also T-2 tetraol, although this analyte possesses a lower toxicity.

The intake of DON mostly depended on the consumption of wheat. In spite of its high consumption, the contribution to the TDI was only 10% assuming the median contamination level. In the bad case scenario, the DON intake amounted to 44% of the TDI (Table 8). The total DON intake by the daily consumed wheat, rye and oat commodities was slightly increased by the contamination of rye flour, while oat flakes as a source for DON-intake were negligible. The contamination of baby food was considerably lower, which resulted in a very low exposure even assuming the bad case scenario.

The exposure to NIV played a minor role after consumption of the cereals examined in this study. Even in the worse-case scenario only about 7% of the t-TDI for NIV were reached. Rye, oats, and baby food did not or hardly contributed to a NIV-exposure (Table 8).

In conclusion, the risk of exceeding the TDI-values could be regarded as low with respect to average consumption and toxin concentrations, in comparison with data of the SCOOP-report (2003). Therein, the percentage of the TDI by the DON-intake of infants ranged between 11% and 96% and between 27% and 560% for the T-2/HT-2 intake, subject to the calculations of the contributing countries. However, infants represented the sub-population with the highest risk to exceed the t-TDI of T-2/HT-2 also in this study. Thus, the setting of a maximum level may be appropriate particularly for baby food or special commodities like oat flakes. It should be noted that wheat, for example, did not contain only DON but also T-2/HT-2 and that the simultaneous occurrence of these toxins leads to a higher total mycotoxin ingestion. Hence, the consideration of a group-TDI for trichothecenes may be useful for a proper risk assessment (Pronk et al. 2002).

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Table 1. MRM-transitions and MS parameters for type A, B, and D trichothecenes

Analyte	MW (g/mol)	Precursor ion		Product ions		DP (V)	CE (eV)	CXP (V)	Dwell time (msec)
Type A trichothecenes									
T-2	466	[M+NH$_4$]$^+$	483.9	Quantifier	305.2	61	19	20	75
				Qualifier	215.2	61	25	14	75
13C-T-2	490	[M+NH$_4$]$^+$	508.3	Quantifier	229.2	46	25	14	75
				Qualifier	322.2	46	19	20	75
HT-2	424	[M+NH$_4$]$^+$	442.4	Quantifier	263.2	51	17	16	75
				Qualifier	215.1	51	19	16	75
T-2 triol	382	[M+NH$_4$]$^+$	400.4	Quantifier	281.2	36	17	20	75
				Qualifier	215.1	36	13	18	75
T-2 tetraol	298	[M+NH$_4$]$^+$	316.2	Quantifier	215.2	36	13	20	150
				Qualifier	281.3	36	11	18	150
DAS	366	[M+NH$_4$]$^+$	384.2	Quantifier	307.3	51	17	10	75
				Qualifier	247.1	51	19	24	75
MAS	324	[M+NH$_4$]$^+$	342.2	Quantifier	265.1	41	13	16	125
				Qualifier	107.1	41	19	10	125
NEO	382	[M+NH$_4$]$^+$	400.2	Quantifier	305.3	51	17	18	125
				Qualifier	245.1	51	25	16	125
DacVOL	350	[M+NH$_4$]$^+$	368.3	Quantifier	291.2	51	13	20	75
				Qualifier	249.1	51	21	18	75
VOL	266	[M+NH$_4$]$^+$	284.2	Quantifier	267.2	36	7	16	125
				Qualifier	249.3	36	11	24	125
Type B trichothecenes									
DON	296	[M-H]$^-$	294.9	Quantifier	265.0	-45	-16	-15	150
				Qualifier	138.0	-45	-22	-11	150
13C-DON	311	[M-H]$^-$	310.3	Quantifier	144.9	-55	-22	-7	150
				Qualifier	279.0	-55	-16	-13	150
3-acDON	338	[M+NH4]$^+$	356.3	Quantifier	231.1	36	25	16	125
				Qualifier	203.0	36	21	22	125
15-acDON	338	[M+NH4]$^+$	356.1	Quantifier	321.1	46	19	28	125
				Qualifier	137.2	46	21	14	125
NIV	312	[M+HCOO]$^-$	357.1	Quantifier	281.0	-50	-18	-13	150
				Qualifier	202.8	-50	-24	-11	150
FX	354	[M+NH4]$^+$	372.1	Quantifier	355.1	41	11	24	125
				Qualifier	247.2	41	19	16	125

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Type D trichothecenes									
SG	544	[M+NH4]⁺	562.3	Quantifier	231.2	51	23	16	75
				Qualifier	249.2	51	21	18	75
SH	528	[M+NH4]⁺	546.5	Quantifier	529.2	36	21	20	75
				Qualifier	281.1	36	21	18	75
RA	532	[M+NH4]⁺	550.5	Quantifier	249.2	56	23	18	175
				Qualifier	533.4	56	15	18	175
VA	502	[M+NH4]⁺	520.4	Quantifier	249.1	51	23	18	175
				Qualifier	457.3	51	17	14	175

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Table 2: Performance parameters of the LC-MS/MS-method

Analyte	Linearity[*] (ng ml^{-1})	Correlation r (calibration points)	RSD (n=5) 1 ng ml^{-1}	RSD (n=5) 10 ng ml^{-1}	DIN 32645[**] LOD (ng ml^{-1})	LOQ (ng ml^{-1})	Signal-to-noise[***] LOD (ng ml^{-1})	LOQ (ng ml^{-1})
Type A trichothecenes								
T-2	LOD – 250	0.9998 (15)	1.8%	2.2%	0.20	0.66	0.026	0.078
HT-2	LOD – 200	0.9995 (16)	3.3%	1.6%	0.32	1.1	0.061	0.18
T-2 triol	LOD – 250	0.9996 (15)	6.7%	4.4%	0.27	0.92	0.16	0.49
T-2 tetraol	LOD – 250	0.9992 (14)	6.5%	2.9%	0.41	1.4	0.10	0.30
DAS	LOD – 250	0.9999 (15)	4.1%	1.8%	0.085	0.29	0.030	0.089
MAS	LOD – 250	0.9999 (16)	2.2%	1.3%	0.085	0.29	0.012	0.035
NEO	LOD – 250	0.9999 (16)	3.8%	0.5%	0.11	0.35	0.029	0.087
DacVOL	LOD – 250	0.9999 (15)	3.1%	1.0%	0.064	0.22	0.017	0.051
VOL	LOD – 200	0.9995 (15)	7.6%	0.8%	0.34	1.1	0.27	0.82
Type B trichothecenes								
DON	LOD – 250	0.9999 (16)	2.8%	1.4%	0.092	0.31	0.038	0.11
3-acDON	LOD – 250	0.9996 (15)	8.4%	1.5%	0.28	0.96	0.14	0.42
15-acDON	LOD – 250	0.9999 (16)	4.7%	2.3%	0.14	0.46	0.033	0.10
NIV	LOD – 250	0.9980 (10)	11%	1.1%	0.70	2.2	1.2	3.6
FX	LOD – 250	0.9999 (16)	4.8%	1.1%	0.11	0.37	0.12	0.35
Type D trichothecenes								
SG	LOD – 250	0.9993 (15)	5.3%	1.4%	0.28	0.95	0.038	0.11
SH	LOD – 200	0.9993 (15)	9.1%	3.1%	0.38	1.3	0.15	0.46
RA	LOD – 250	0.9995 (15)	3.9%	1.7%	0.22	0.74	0.021	0.063
VA	LOD – 250	0.9996 (16)	2.5%	0.7%	0.20	0.67	0.044	0.13

[*]calculated using F-test (Mandel) [**]uncertainty 33.3 %; probability of error 1 %; n=1 [***]used for quantification; LOD: S/N=3, LOQ: S/N=9

Table 3: Average recovery rates of type A, B and D trichothecenes from wheat flour, rye

flour, and oat flakes (fortification levels 1 µg kg^{-1}, 10 µg kg^{-1}, 50 µg kg^{-1}; n=5)

Analyte	Average recovery rate (%) ± RSD (%)		
	wheat flour	rye flour	oat flakes
Type A trichothecenes			
T-2	108 ± 2.8	95 ± 6.1	103 ± 2.7
HT-2	98 ± 4.9	93 ± 10	102 ± 3.4
T-2 triol	97 ± 5.0	92 ± 3.7	99 ± 4.8
T-2 tetraol	73 ± 7.8	75 ± 8.4	60 ± 8.4
DAS	106 ± 1.3	101 ± 3.6	107 ± 2.6
MAS	101 ± 1.5	85 ± 5.4	97 ± 2.3
NEO	99 ± 5.0	90 ± 6.8	98 ± 3.3
DacVOL	96 ± 2.8	86 ± 3.6	96 ± 3.6
VOL	101 ± 5.3	83 ± 8.7	95 ± 6.8
Type B trichothecenes			
DON	92 ± 17	89 ± 11	98 ± 4.5
3-acDON	104 ± 5.0	96 ± 6.2	99 ± 4.6
15-acDON	98 ± 6.0	95 ± 6.4	102 ± 3.8
NIV	61 ± 16	67 ± 11	67 ± 8.7
FX	87 ± 4.9	84 ± 9.5	94 ± 4.7
Type D trichothecenes			
SG	91 ± 4.8	83 ± 6.3	98 ± 6.1
SH	87 ± 5.3	85 ± 5.8	92 ± 6.0
RA	107 ± 4.2	87 ± 6.9	89 ± 12
VA	78 ± 4.9	76 ± 5.3	81 ± 9.0

Table 4. Trueness assessment of the method by interlaboratory tests and comparison measurements for T-2, HT-2, and DON

Laboratory / method	Material	Analyte	Results	Results with this LC-MS/MS method	Accuracy
- Interlaboratory test (DLA Ahrensburg, Germany; 28 participating labs)	Cereals for animal feed (DLA-05/2007)	DON	$257 - 555$ µg kg^{-1} (X = 405 µg kg^{-1})	324 µg kg^{-1}	z-score = -1.1
- Comparison measurement (Chair of Food Chemistry, Technische Universitaet Muenchen, Germany; stable isotope dilution assay - LC-MS/MS)	Oat flakes (1)	T-2	20 µg kg^{-1}	18 µg kg^{-1}	90%
		HT-2	50 µg kg^{-1}	49 µg kg^{-1}	98%
	Oat flakes (2)	T-2	20 µg kg^{-1}	17 µg kg^{-1}	85%
		HT-2	45 µg kg^{-1}	44 µg kg^{-1}	98%
- Comparison measurement (Bavarian Health and Food Safety Authority, Oberschleißheim, Germany; HPLC-FLD)	Wheat flour	DON	106 µg kg^{-1}	113 µg kg^{-1}	107%
	Whole wheat flour	DON	130 µg kg^{-1}	121 µg kg^{-1}	93%
	Wheat kernels	DON	158 µg kg^{-1}	149 µg kg^{-1}	94%
	Wheat bran (1)	DON	294 µg kg^{-1}	325 µg kg^{-1}	111%
	Wheat bran (2)	DON	1087 µg kg^{-1}	1069 µg kg^{-1}	98%

Table 5. Rates and levels of contamination of different commodities of wheat with the most relevant trichothecenes. All samples (n=130), wheat flour (n=39), whole wheat flour (n=11), wheat kernels (n=52), semolina (n=13), wheat bran (n=10), and wheat-containing infant food (n=5)

Analyte	Commodity	Contamination rate (%)	Mean (μg kg^{-1})	Median (μg kg^{-1})	95th percentile (μg kg^{-1})	Maximum value (μg kg^{-1})
T-2	all wheat samples	85	0.21	0.09	0.91	1.9
	wheat flour	85	0.09	0.04	0.17	1.2
	whole wheat flour	100	0.16	0.15	0.25	0.25
	wheat kernels	94	0.27	0.17	0.76	1.1
	semolina	39	0.02	< LOD	0.06	0.08
	wheat bran	100	0.84	0.60	1.8	1.9
	infant food	40	0.04	< LOD	0.13	0.15
HT-2	all wheat samples	94	1.6	0.84	5.6	22
	wheat flour	97	0.65	0.32	0.92	11
	whole wheat flour	100	1.4	1.6	2.1	2.2
	wheat kernels	100	1.9	1.4	4.4	5.8
	semolina	54	0.13	0.09	0.45	0.67
	wheat bran	100	7.1	6.7	17	22
	infant food	80	0.55	0.32	1.2	1.3
T-2 + HT-2	all wheat samples	94	1.8	0.91	6.4	24
	wheat flour	97	0.74	0.34	1.1	12
	whole wheat flour	100	1.6	1.8	2.4	2.4
	wheat kernels	100	2.2	1.9	5.2	6.8
	semolina	54	0.15	0.09	0.50	0.74
	wheat bran	100	7.9	7.6	19	24
	infant food	80	0.59	0.36	1.3	1.3
T-2 triol	all wheat samples	18	0.08	< LOD	0.25	1.4
	wheat flour	2.6	0.03	< LOD	< LOD	1.4
	whole wheat flour	9.1	0.02	< LOD	0.12	0.25
	wheat kernels	28	0.07	< LOD	0.25	0.25
	semolina	n.d.	< LOD	< LOD	< LOD	< LOD
	wheat bran	78	0.55	0.52	1.2	1.3
	infant food	n.d.	< LOD	< LOD	< LOD	< LOD
T-2 tetraol	all wheat samples	53	2.6	0.15	8.0	83
	wheat flour	21	0.78	< LOD	1.4	25
	whole wheat flour	73	1.5	1.7	3.2	3.4

	wheat kernels	79	2.7	1.8	8.0	10
	semolina	7.7	0.04	< LOD	0.19	0.46
	wheat bran	100	17	4.2	65	83
	infant food	20	0.03	< LOD	0.12	0.15
DAS	all wheat samples	3.1	0.01	< LOD	< LOD	0.25
	wheat flour	2.6	0.01	< LOD	< LOD	0.25
	whole wheat flour	n.d.	< LOD	< LOD	< LOD	< LOD
	wheat kernels	1.9	< LOD	< LOD	< LOD	0.25
	semolina	n.d.	< LOD	< LOD	< LOD	< LOD
	wheat bran	22	0.05	< LOD	0.25	0.25
	infant food	n.d.	< LOD	< LOD	< LOD	< LOD
MAS	all wheat samples	92	0.30	0.11	0.88	5.9
	wheat flour	92	0.11	0.06	0.19	1.6
	whole wheat flour	100	0.55	0.51	1.3	1.4
	wheat kernels	98	0.27	0.21	0.67	0.86
	semolina	62	0.02	0.02	0.05	0.06
	wheat bran	100	1.6	0.83	4.9	5.9
	infant food	80	0.04	0.02	0.11	0.12
NEO	all wheat samples	24	0.02	< LOD	0.11	0.40
	wheat flour	2.6	< LOD	< LOD	< LOD	0.17
	whole wheat flour	9.1	< LOD	< LOD	0.02	0.04
	wheat kernels	40	0.03	< LOD	0.12	0.15
	semolina	n.d.	< LOD	< LOD	< LOD	< LOD
	wheat bran	89	0.10	0.04	0.33	0.40
	infant food	n.d.	< LOD	< LOD	< LOD	< LOD
DON	all wheat samples	100	57	23	162	1163
	wheat flour	100	45	25	110	613
	whole wheat flour	100	39	13	116	131
	wheat kernels	100	49	27	154	235
	semolina	100	39	17	140	172
	wheat bran	100	225	64	839	1163
	infant food	100	12	9.9	22	24
3-acDON	all wheat samples	52	0.57	0.21	2.4	15
	wheat flour	38	0.29	< LOD	0.77	5.2
	whole wheat flour	64	0.44	0.21	1.5	1.8
	wheat kernels	68	0.68	0.47	2.7	3.8
	semolina	15	0.05	< LOD	0.31	0.47
	wheat bran	67	2.2	0.21	10	15

	infant food	20	0.30	< LOD	1.2	1.5
15-acDON	all wheat samples	72	0.90	0.17	3.5	26
	wheat flour	85	0.42	0.11	0.82	8.8
	whole wheat flour	64	0.60	0.33	1.8	2.1
	wheat kernels	72	1.0	0.46	4.5	6.1
	semolina	46	0.10	< LOD	0.51	0.70
	wheat bran	78	3.8	0.96	17	26
	infant food	60	0.69	0.12	2.6	3.2
NIV	all wheat samples	67	6.8	1.8	24	96
	wheat flour	56	4.3	1.8	12	77
	whole wheat flour	91	13	9.5	41	62
	wheat kernels	77	5.7	1.8	19	31
	semolina	31	3.4	< LOD	17	35
	wheat bran	89	23	14	68	96
	infant food	40	2.1	< LOD	7.4	8.8
FX	all wheat samples	5.4	0.02	< LOD	0.10	0.93
	wheat flour	n.d.	< LOD	< LOD	< LOD	< LOD
	whole wheat flour	n.d.	< LOD	< LOD	< LOD	< LOD
	wheat kernels	11	0.04	< LOD	0.18	0.93
	semolina	7.7	0.01	< LOD	0.07	0.18
	wheat bran	n.d.	< LOD	< LOD	< LOD	< LOD
	infant food	n.d.	< LOD	< LOD	< LOD	< LOD

n.d. = not detectable

Table 6. Rates and levels of contamination of different commodities of rye with the most relevant trichothecenes. All samples (n=61), rye flour (n=15), whole rye flour (n=9), rye kernels (n=37)

Analyte	Commodity	Contamination rate (%)	Mean (μg kg^{-1})	Median (μg kg^{-1})	95th percentile (μg kg^{-1})	Maximum value (μg kg^{-1})
T-2	all samples	87	0.13	0.11	0.30	0.77
	rye flour	100	0.16	0.13	0.33	0.42
	whole rye flour	100	0.22	0.18	0.59	0.77
	rye kernels	78	0.10	0.04	0.29	0.30
HT-2	all samples	93	0.56	0.42	1.5	2.6
	rye flour	100	0.73	0.45	1.8	2.6
	whole rye flour	100	0.76	0.54	1.8	2.2
	rye kernels	89	0.45	0.36	1.1	1.5
T-2 + HT-2	all samples	95	0.69	0.53	1.8	3.1
	rye flour	100	0.89	0.57	2.1	3.1
	whole rye flour	100	1.0	0.74	2.4	3.0
	rye kernels	92	0.55	0.51	1.4	1.8
T-2 triol	all samples	4.7	0.01	< LOD	< LOD	0.25
	rye flour	6.3	0.02	< LOD	0.06	0.25
	whole rye flour	13	0.03	< LOD	0.16	0.25
	rye kernels	2.9	0.01	< LOD	< LOD	0.25
T-2 tetraol	all samples	79	2.3	2.0	6.4	8.1
	rye flour	88	2.5	2.3	6.3	6.4
	whole rye flour	50	2.1	0.68	6.8	7.4
	rye kernels	81	2.3	1.9	6.4	8.1
DAS	all samples	n.d.	< LOD	< LOD	< LOD	< LOD
	rye flour	n.d.	< LOD	< LOD	< LOD	< LOD
	whole rye flour	n.d.	< LOD	< LOD	< LOD	< LOD
	rye kernels	n.d.	< LOD	< LOD	< LOD	< LOD
MAS	all samples	87	0.05	0.02	0.15	0.31
	rye flour	94	0.05	0.03	0.14	0.20
	whole rye flour	88	0.07	0.02	0.24	0.31
	rye kernels	84	0.04	0.02	0.13	0.18
NEO	all samples	15	0.01	< LOD	0.04	0.10
	rye flour	19	0.01	< LOD	0.04	0.04
	whole rye flour	38	0.02	< LOD	0.08	0.10
	rye kernels	8.1	< LOD	< LOD	0.04	0.04

DON	all samples	100	28	15	92	288
	rye flour	100	26	23	46	64
	whole rye flour	100	32	6.5	105	112
	rye kernels	100	28	14	79	288
3-acDON	all samples	59	0.39	0.21	1.6	5.0
	rye flour	63	0.19	0.21	0.54	0.63
	whole rye flour	50	0.49	0.11	2.0	2.4
	rye kernels	59	0.45	0.21	1.6	5.0
15-acDON	all samples	80	0.73	0.35	2.6	8.6
	rye flour	100	0.43	0.37	0.86	0.99
	whole rye flour	63	0.88	0.20	3.5	4.3
	rye kernels	76	0.84	0.35	2.7	8.6
NIV	all samples	3.3	0.06	< LOD	< LOD	1.8
	rye flour	n.d.	< LOD	< LOD	< LOD	< LOD
	whole rye flour	n.d.	< LOD	< LOD	< LOD	< LOD
	rye kernels	5.4	0.10	< LOD	0.36	1.8
FX	all samples	1.6	0.01	< LOD	< LOD	0.18
	rye flour	n.d.	< LOD	< LOD	< LOD	< LOD
	whole rye flour	n.d.	< LOD	< LOD	< LOD	< LOD
	rye kernels	2.7	< LOD	< LOD	< LOD	0.18

n.d. = not detectable

Table 7. Rates and levels of contamination of different commodities of oats with the most relevant trichothecenes: All samples (n=98), fine oat flakes (n=31), oat flakes (n=23), oat kernels (n=19), oat bran (n=12), and oat-containing infant food (n=13)

Analyte	Commodity	Contamination rate (%)	Mean ($\mu g\ kg^{-1}$)	Median ($\mu g\ kg^{-1}$)	95th percentile ($\mu g\ kg^{-1}$)	Maximum value ($\mu g\ kg^{-1}$)
T-2	all samples	100	4.2	2.2	15	34
	fine oat flakes	100	6.7	3.6	20	34
	oat flakes	100	3.8	1.8	11	17
	oat kernels	100	2.5	1.5	7.3	12
	oat bran	100	4.0	3.1	9.2	11
	infant food	100	1.8	1.3	4.7	5.6
HT-2	all samples	99	10	6.0	35	51
	fine oat flakes	100	14	8.3	44	51
	oat flakes	100	9.6	5.4	24	48
	oat kernels	100	7.6	4.4	18	34
	oat bran	100	11	8.5	25	32
	infant food	92	6.1	4.7	15	19
T-2 + HT-2	all samples	100	15	8.2	50	85
	fine oat flakes	100	21	12	63	85
	oat flakes	100	13	7.2	33	65
	oat kernels	100	10	6.0	25	46
	oat bran	100	15	12	34	43
	infant food	100	7.9	6.0	20	23
T-2 triol	all samples	60	0.43	0.25	1.5	2.7
	fine oat flakes	74	0.60	0.55	1.5	2.7
	oat flakes	52	0.39	0.25	1.4	1.5
	oat kernels	53	0.33	0.25	1.2	1.5
	oat bran	58	0.38	0.25	1.2	1.5
	infant food	54	0.26	0.25	0.85	1.3
T-2 tetraol	all samples	90	9.7	6.4	25	85
	fine oat flakes	94	11	9.5	22	34
	oat flakes	87	7.7	5.2	26	27
	oat kernels	95	8.4	5.3	24	24
	oat bran	100	11	10	26	26
	infant food	69	12	3.0	44	85
DAS	all samples	60	0.05	0.04	0.17	0.38
	fine oat flakes	71	0.08	0.04	0.19	0.38

	oat flakes	65	0.04	0.04	0.12	0.17
	oat kernels	47	0.04	< LOD	0.17	0.25
	oat bran	75	0.04	0.04	0.08	0.12
	infant food	31	0.03	< LOD	0.12	0.25
MAS	all samples	95	0.09	0.07	0.23	0.66
	fine oat flakes	97	0.59	0.28	1.9	2.7
	oat flakes	91	0.06	0.06	0.12	0.18
	oat kernels	89	0.09	0.05	0.25	0.27
	oat bran	100	0.08	0.06	0.16	0.17
	infant food	92	0.07	0.06	0.20	0.21
NEO	all samples	92	0.37	0.22	1.4	2.7
	fine oat flakes	97	0.59	0.28	1.9	2.7
	oat flakes	96	0.33	0.14	1.1	1.4
	oat kernels	79	0.22	0.13	0.64	0.9
	oat bran	100	0.30	0.26	0.62	0.81
	infant food	85	0.16	0.09	0.47	0.51
DON	all samples	74	2.8	0.53	11	55
	fine oat flakes	87	4.3	1.2	18	55
	oat flakes	70	2.6	0.30	8.9	39
	oat kernels	84	2.9	1.1	11	30
	oat bran	58	0.67	0.18	2.6	4.2
	infant food	54	1.1	0.17	4.7	6.5
3-acDON	all samples	28	0.43	< LOD	2.1	8.2
	fine oat flakes	48	0.97	< LOD	5.8	8.2
	oat flakes	13	0.27	< LOD	1.9	3.6
	oat kernels	16	0.19	< LOD	1.4	1.9
	oat bran	25	0.12	< LOD	0.58	0.75
	infant food	23	0.09	< LOD	0.44	0.78
15-acDON	all samples	28	0.11	<LOD	0.63	1.4
	fine oat flakes	32	0.13	< LOD	0.77	1.3
	oat flakes	8.7	0.03	< LOD	0.04	0.58
	oat kernels	42	0.13	< LOD	0.62	0.75
	oat bran	25	0.08	< LOD	0.35	0.38
	infant food	31	0.20	< LOD	0.88	1.4
NIV	all samples	24	0.96	< LOD	6.1	17
	fine oat flakes	39	2.0	< LOD	7.7	17
	oat flakes	17	0.31	< LOD	1.8	1.8
	oat kernels	26	1.1	< LOD	6.4	8.7

	oat bran	17	0.30	< LOD	1.8	1.8
	infant food	7.7	0.14	< LOD	0.72	1.8
FX	all samples	6.1	0.02	< LOD	0.18	0.53
	fine oat flakes	6.4	0.01	< LOD	0.09	0.18
	oat flakes	8.7	0.04	< LOD	0.41	0.53
	oat kernels	5.3	0.01	< LOD	0.02	0.18
	oat bran	n.d.	< LOD	< LOD	< LOD	< LOD
	infant food	7.7	0.01	< LOD	0.07	0.18

n.d. = not detectable

Table 8. Estimated intake of DON, NIV and T-2/HT-2 by average consumption of selected food

commodities by two- to five-year-old children[*] and nine-month-old infants[**]

| Commodity	Average consumption (g/day)	Estimated toxin intake (ng/day) [% TDI]					
		DON		NIV		T-2/HT-2	
		mean case	bad case	mean case	bad case	mean case	bad case
Wheat flour	65.2[*]	1630 [10]	7172 [44]	117 [1.0]	782 [6.9]	22 [2.3]	72 [7.4]
Rye flour	12.3[*]	283 [1.8]	566 [3.5]	0 [0]	0 [0]	7.0 [0.72]	26 [2.7]
Fine oat flakes	3.1[*]	3.7 [0.02]	56 [0.35]	0 [0]	24 [0.21]	37 [3.8]	195 [20]
Oat-containing infant food	30.1[**]	5.1 [0.06]	141 [1.6]	0 [0]	22 [0.35]	181 [34]	602 [112]

[*] Banasiak et al. 2005 [**] Kersting et al. 1998
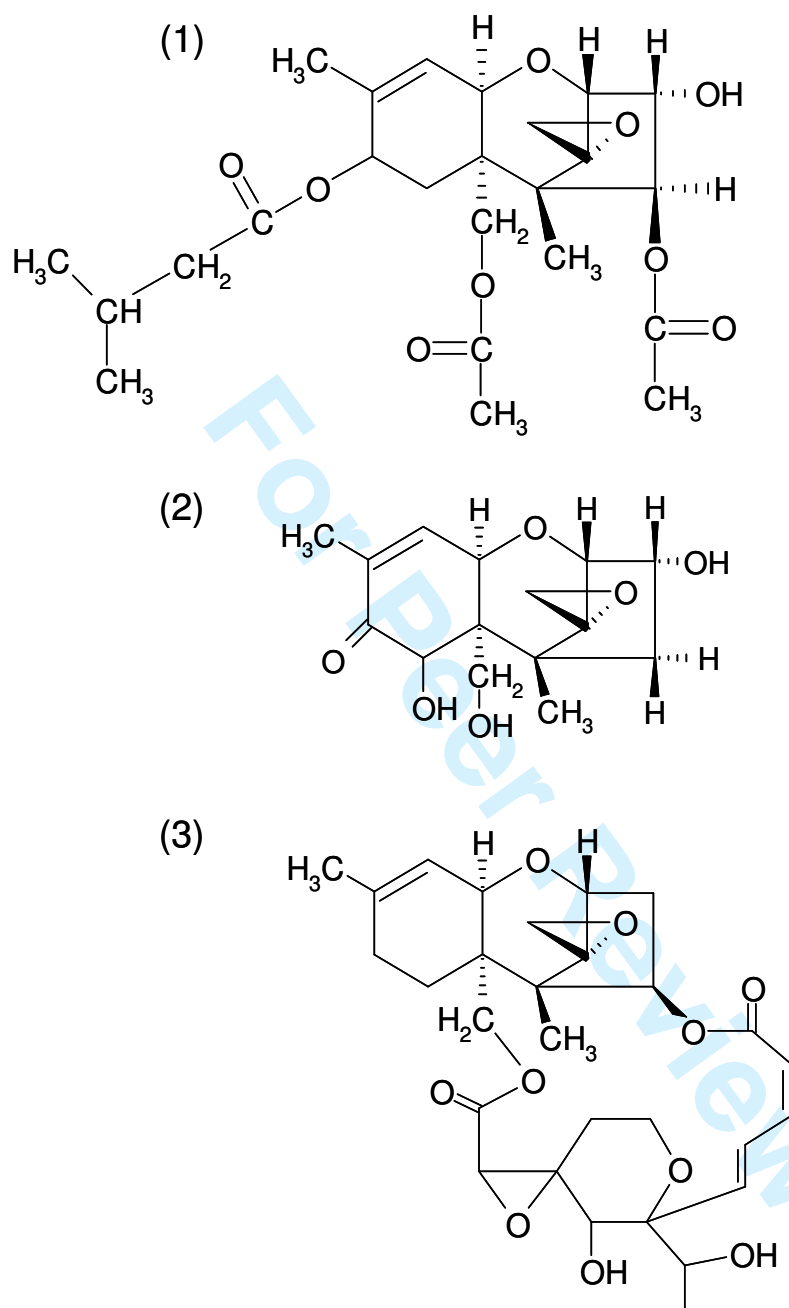
Figure 1. Examples of different structures of trichothecenes; (1) type A: T-2 toxin; (2) type B: DON;
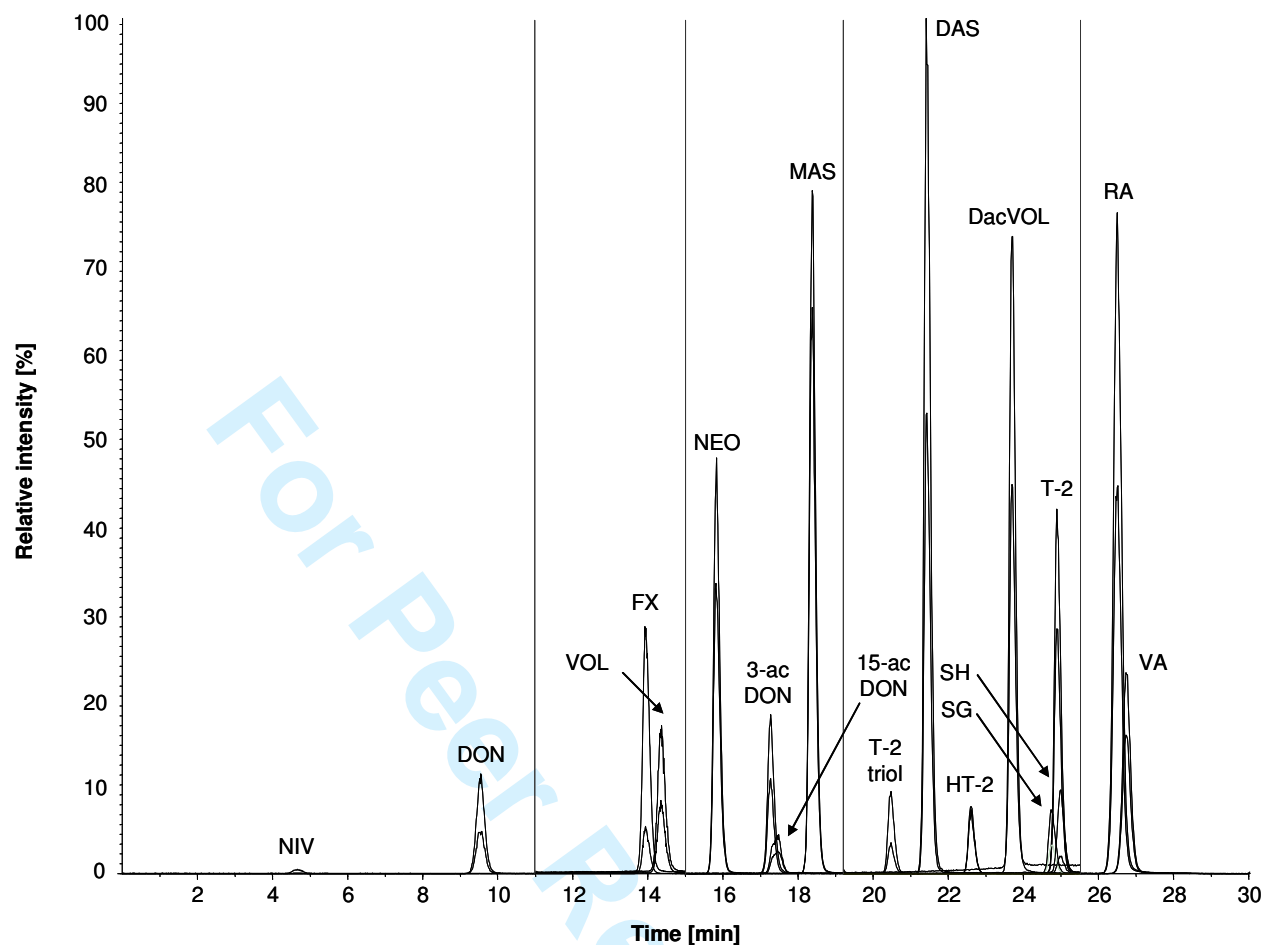
(3) type D: satratoxin G (Ueno 1983a)

Figure 2. LC-MS/MS chromatogram (separated in five periods) of a multi-trichothecene standard (10 ng ml^{-1}) with two mass transitions per toxin (quantifier and qualifier)
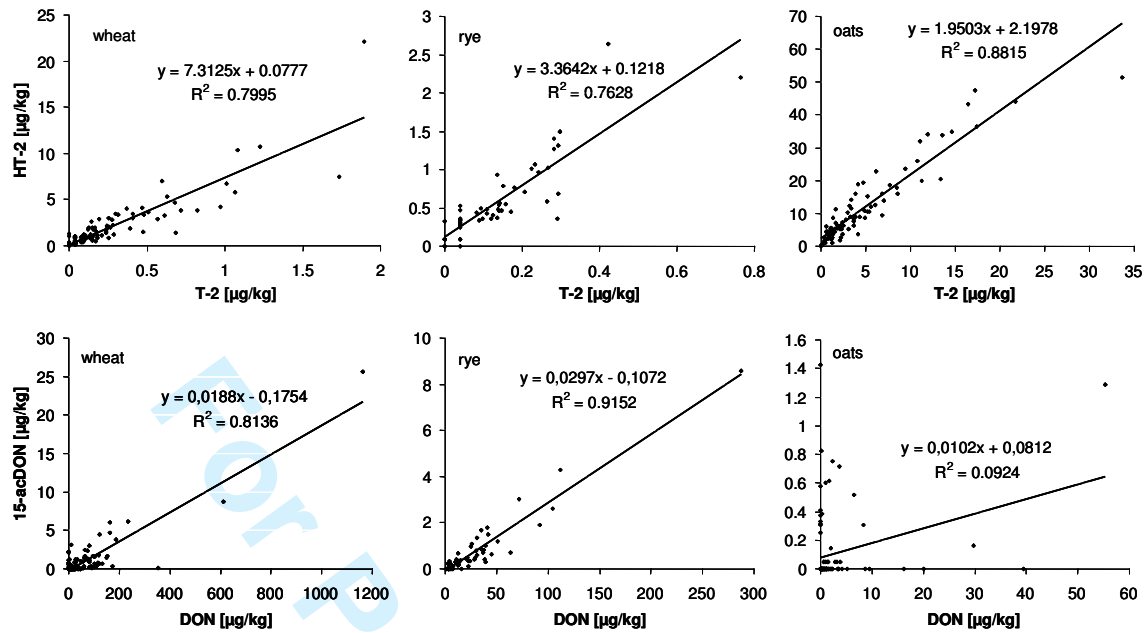
Figure 3. Correlation between T-2 and HT-2 levels (above) and DON and 15-acDON levels (below) in wheat samples (n=130), rye samples (n=61), and oat samples (n=98)
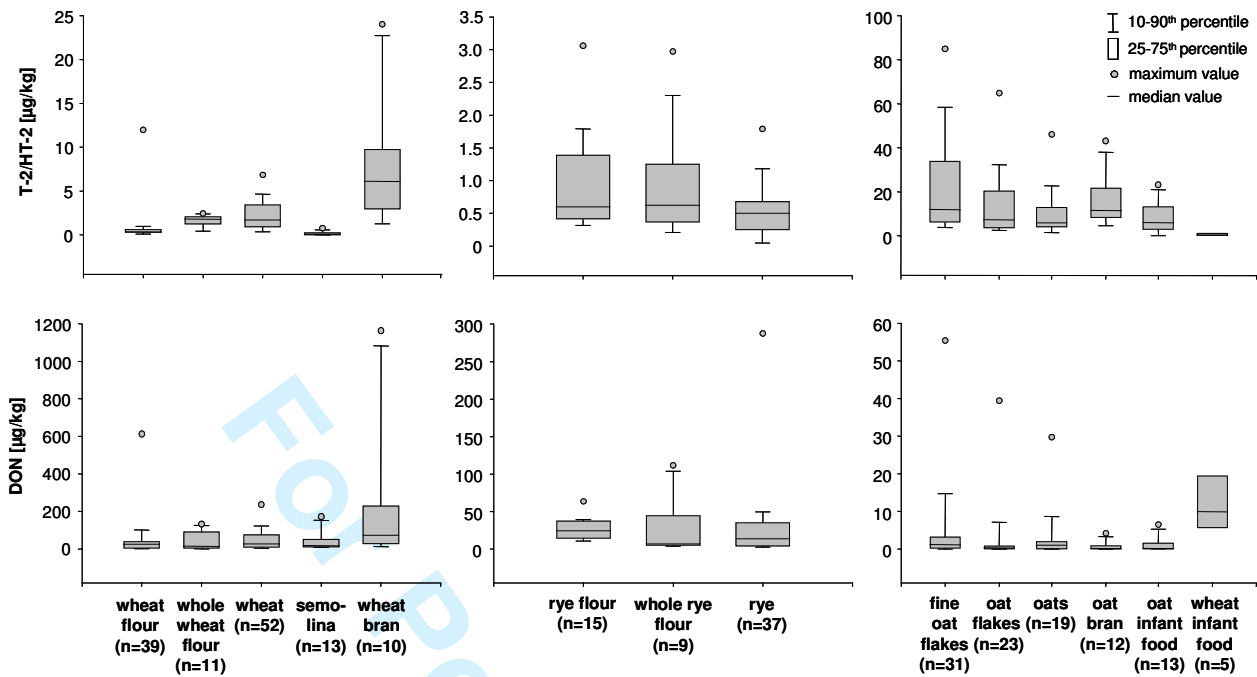
Figure 4. Comparison of contamination levels of T-2/HT-2 and DON in different food commodities of wheat, rye and oats