

## SCIENTIFIC / TECHNICAL REPORT submitted to EFSA

### Occurrence data of trichothecene mycotoxins T-2 toxin and HT-2 toxin in food and feed<sup>1</sup>

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#### Summary

T-2 toxin and HT-2 toxin are mycotoxins that are produced by several *Fusarium* species in cereal grains. They may contaminate the harvest grain and feed and food products thereof, and - after ingestion - affect animal and human health.

The aim of the current study was to collect, compile and synthesise data on T-2 toxin and HT-2 toxin, including contamination of food and feed commodities and products thereof in Europe, co-occurrence with other mycotoxins, and factors (environmental, agronomic and processing) influencing the levels of T-2 toxin and HT-2 toxin in plant products used for food and feed production.

The available data show that oats can be highly contaminated with T-2 toxin and HT-2 toxin, with frequently high incidence and concentration. Particularly, contamination seems to have increased in 2005 and some following years, at least in the Scandinavian countries. The occurrence and concentration of T-2 toxin and HT-2 toxin in barley has increased across Europe since 2004, and stabilized at an incidence of about 80%. Contamination in the resulting malt used for beer production has also increased. In some occasions, maize is contaminated with T-2 toxin and HT-2 toxin, usually at a moderate level. T-2 toxin and HT-2 toxin contamination of wheat occurs very infrequently and at a low concentration level. Feed products that can be highly contaminated include by-products from oat

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processing (pellets). Food products generally show low incidence and concentration of T-2 toxin and HT-2 toxin, however, oat products may contain the two mycotoxins.

In commodities, T-2 toxin and HT-2 toxin are highly correlated to each other with the level of HT-2 toxin being between nearly two to seven times higher than T-2 toxin. In food products, the relationship between T-2 toxin and HT-2 toxin is weak or even absent. T-2 toxin and HT-2 toxin seem to have no correlation with deoxynivalenol and nivalenol in cereals. Field factors that influence T-2 toxin and HT-2 toxin include region-year (climate), variety, sowing date, pre-crop, and organic production. Fungicides seem to have no or only a weak effect. Processing cereals will substantially reduce T-2 toxin and HT-2 toxin contamination in food products due to redistribution over the various cereal fractions. The toxin levels increase in the by-products, which are often used for animal feeding.

The results of this study may be used in risk assessments on T-2 toxin and HT-2 toxin at the European level, such to establish maximum permissible limits in feed and food products and/or their commodities.

**Key words: trichothecenes, T-2 toxin, HT-2 toxin, *Fusarium*, cereals**

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## Background

Undesirable substances such as mycotoxins can be present in plants and products derived thereof. Depending on the nature and concentration of these compounds, they may be of concern for human and/or animal health. The mycotoxins T-2 toxin (T-2) and HT-2 toxin (HT-2) are type A trichothecenes which, in general, are more toxic than type B trichothecenes (e.g. deoxynivalenol). Risk assessments on T-2 and HT-2 at the European level have not been done. It is expected that the European Commission will ask EFSA to assess the risks to human and animal health related to these substances in the near future. To carry out these risk assessments to the highest standards and in an efficient way, scientific background information on T-2 and HT-2 is needed.

## Terms of reference

The outcome of the project NP/EFSA/UNIT CONTAM/2010/01 shall contain:

Collection, compilation, and synthesis of scientific information on the occurrence (incidence and concentration) of T-2 and HT-2 in food and feed in Europe. The key findings, including data on both mycotoxins in the two areas listed below, shall be written down in a scientific report. The emphasis is on data from the year 2000 onwards. The report shall contain separate chapters for T-2 and HT-2, with both chapters including data on food and feed, separately. A full reference list according to the EFSA citation standards shall be included. Additionally, all scientific publications cited will be stored in an Endnote database.

Scientific information shall be collected, compiled and synthesised for T-2 and HT-2 for the following two areas:

Area 1: Occurrence and concentration data in food and feed commodities in Europe, particularly to identify food and feed commodities which are considered as susceptible for contamination by T-2 and HT-2, including co-occurrence data on other mycotoxins and data on carry-over of T-2 and HT-2 from feed to food products, if applicable. An indication of trends of the concentrations of T-2 and HT-2 in food and feed should be included.

Area 2: Factors - including environmental, agronomic and processing factors - influencing the levels of T-2 and HT-2 in plant products used for food and feed production, including possible mitigation.

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## 1. Introduction and Objectives

Trichothecenes are a large family of chemically related mycotoxins produced by various fungal species. They are mainly produced by *Fusarium* species in cereal grains and can result in contamination of the harvested grain and feed and food products thereof. Trichothecene mycotoxins may affect animal and human health as they have an immunosuppressive effect due to their multiple inhibitory effects on eukaryotic cells, including inhibition of protein, DNA and RNA synthesis, inhibition of mitochondrial function, and effects on cell division and membrane function (Rocha et al., 2005). They may cause neural disturbance, haemorrhages, skin irritation, vomiting, diarrhoea, weight loss, reduced feed intake and reduced production of milk and eggs in experimental animals and livestock (Richard, 2007).

All trichothecenes share the cyclic sesquiterpene structure but differ in the type of functional group attached to the carbon backbone. Based on their structure, the group of trichothecenes can be divided into two types, named type A and B (See Appendix).

Most *Fusarium* species produce either type A or type B trichothecenes. The most common trichothecenes belong to type A (T-2 toxin, HT-2 toxin, 4,15-diacetoxyscirpenol) and type B (deoxynivalenol, 3-acetyl deoxynivalenol, 15-acetyl deoxynivalenol, nivalenol). In general, toxins of the trichothecenes type A group are much more toxic than those of the type B group. Two of the most toxic members of the group of trichothecenes mycotoxins are HT-2 toxin (HT-2) and T-2 toxin (T-2), two closely related type A trichothecenes. As T-2 is metabolised into HT-2 after ingestion, they are considered to have equal toxicity (Canada et al., 2001).

T-2 and HT-2 can be produced by several *Fusarium* species, including *F. poae*, *F. sporotrichioides*, *F. armeniacum* (syn. *F. acuminatu* subspecies *armeniaceum*) and the recently identified *F. Langsethiae* (Logrieco et al., 2003; Torp and Nirenberg, 2004). Torp and Nirenberg (2004) describe, illustrate and discuss this *Fusarium* species, which morphologically resembles *F. poae*. *F. Langsethiae* was first identified as new species in Norway, with surveys reporting the regularly occurrence of T-2 and HT-2 in oats (Kosiak et al., 1997; Langseth and Rundberget, 1999). This new species was also found in grains in other countries in Europe (Torp and Nirenberg, 2004). *F. Langsethiae* and *F. sporotrichioides* are thought to be the main producers of T-2 and HT-2 (Kosiak et al., 1997; Thrane et al., 2004). In addition to the four *Fusarium* species mentioned, Visconti and Pascale (2010) also mention *F. sambucinum* as producer of T-2 and HT-2.

As far as concerns trichothecene mycotoxins in cereals and products thereof, the EC has set maximum permissible limits for deoxynivalenol (DON) only, with varying levels depending on the product (Regulation EC/1881/2006).

Limits for T-2 and HT-2 in food are timetabled for the near future (Edwards et al., 2009). However, no recent risk assessments on T-2 and HT-2 have not been done at the European level, mainly because of (to date) a lack of a clear overview of the information on their occurrence has not allowed the risk assessment required prior to deciding appropriate limits. The toxicity of T-2 and HT-2 was evaluated by the Scientific Committee for Food (SCF) of the European Commission. It was considered to be significantly higher than other *Fusarium* mycotoxins on the basis of their general toxicity, hemotoxicity and immunotoxicity (SCF, 2001). This had led to the establishment of a temporary tolerable daily intake (TDI) of 0.06 µg kg<sup>-1</sup> of body weight per day for T-2 and HT-2 combined (SCF 2001). To carry out these risk assessments to the highest standards and in an efficient way, scientific background information on T-2 and HT-2 is needed.

The objective of the current study was to collect, compile, and evaluate scientific information on the occurrence of T-2 and HT-2 in food and feed commodities and products thereof in Europe, on factors that affect the levels of T-2 and HT-2 in plant products used for food and feed production (including environmental, agronomic and processing conditions), as well as on co-occurrence of T-2 and HT-2 with other mycotoxins.

## 2. Materials and Methods

Scientific information has been collected, compiled and synthesised for the following two areas related to T-2 and HT-2:

- Incidence and concentration data in food and feed commodities and products thereof in Europe, and the co-occurrence between T-2 and HT-2 with each other as well as with other mycotoxins;
- Factors - including environmental, agronomic and processing factors – that influence the occurrence of T-2 and HT-2 in plant products used for food and feed production.

A literature search was held to collect the above-mentioned information using the databases SCOPUS and ISI Web of Science. Relevant scientific publications were searched for using the following main key-words: T-2 toxin, HT-2 toxin, trichothecenes, cereals, and *Fusarium*. The emphasis was on published data from the year 2000 onwards, however, if deemed of added value, older data has been included. The resulting publications were evaluated for their relevance. Furthermore, information from

the two European Commission Fusarium Forum meetings held in 2008 and 2009 was used. Additional information/data was retrieved from scientific researchers, if necessary.

### **3. Results and discussion**

Reports of T-2 and HT-2 are mainly restricted to cereal grains in Europe. Within Europe, most reports for the two toxins come from the Scandinavian countries, France and the UK. The limited reports originating from these countries might be related to the survey and analytical methods used. It can not be concluded that these toxins will not occur in other European countries. The results of the occurrence of T-2 and HT-2 are reported in the following sections (sections 3.1 and 3.2) for the two toxins, separately, and summarized in Tables 1 and 2.

#### **3.1 Occurrence of T-2 toxin**

##### **3.1.1 Field and commodities**

In the UK, the occurrence of T-2 and HT-2 in wheat, barley and oats in the period 2001-2005 has been investigated (Edwards, 2009a; Edwards 2009b; Edwards 2009c). Samples were taken just after harvest, in the combine or trailer. The number of samples taken were 1624 for wheat, 446 for barley, and 458 for oats (no samples in 2001). At the level of detection (LOD) of 10  $\mu\text{g kg}^{-1}$ , T-2 was found in 16% of the wheat samples and 12% of the barley samples. For both wheat and barley, the mean and median concentration of all samples were very low, below 10  $\mu\text{g kg}^{-1}$ . In oats, T-2 was detected in 84% of the samples, with a frequently high concentration. The mean, median and maximum concentrations were 84  $\mu\text{g kg}^{-1}$ , 140  $\mu\text{g kg}^{-1}$  and 2406  $\mu\text{g kg}^{-1}$ , respectively.

A four-year study (2004-2007) examined the incidence and concentrations of T-2 and HT-2 in wheat, oats and maize at intake to United Kingdom mills. The total numbers of samples taken were 60 wheat (all UK), 27 oats (21 from UK/Ireland and six from Scandinavia), and 86 maize (56 from France and 30 from Argentina) (Scudamore et al., 2009). At wheat intake, only three out of 57 samples were positive for T-2 (LOD 10  $\mu\text{g kg}^{-1}$ ), with a maximum level of 13  $\mu\text{g kg}^{-1}$ . At intake of oats, T-2 level ranges in the 21 samples of oats grown in the UK/Ireland were: five samples in 20-49  $\mu\text{g kg}^{-1}$ , 14 samples in 50-499  $\mu\text{g kg}^{-1}$ ; 1 sample in 500-999  $\mu\text{g kg}^{-1}$ , and 1 sample of 1610  $\mu\text{g kg}^{-1}$ . T-2 levels in each of the six samples from Scandinavia were between 5-499  $\mu\text{g kg}^{-1}$  (maximum value 221  $\mu\text{g kg}^{-1}$ ).

At intake of the 56 consignments of French maize, T-2 was found in 22 samples, with their level ranges being: 12 samples in 10-19  $\mu\text{g kg}^{-1}$ ; eight samples in 20-49  $\mu\text{g kg}^{-1}$ , two samples in 50-499  $\mu\text{g kg}^{-1}$  (maximum value 107  $\mu\text{g kg}^{-1}$ ).

Langseth and Rundberget (1999) reported T-2 contamination of a total of 449 grain samples from different regions in Norway from 1996-1998 crops. Of the 102 barley samples, 5% was positive for T-2 (LOD 20  $\mu\text{g kg}^{-1}$ ), with the mean of the positive samples being 85  $\mu\text{g kg}^{-1}$ . Of the 178 oat samples, 30% was contaminated with a mean level of the positive samples of 60  $\mu\text{g kg}^{-1}$ . Of the 169 wheat samples, 0.6% was contaminated at an average level of 20  $\mu\text{g kg}^{-1}$ .

Brodal et al. (2008) has presented more recent Norwegian data on T-2 in various cereals in 2006 and 2007. In 2006, T-2 levels in oats were between ND (not detected; level not specified) – 350  $\mu\text{g kg}^{-1}$ ; in spring wheat T-2 ranged between ND – 130  $\mu\text{g kg}^{-1}$ , and in barley (few samples) T-2 ranged between ND – LOQ (level of quantification; level not specified). In 2007, these T-2 ranges were ND -190  $\mu\text{g kg}^{-1}$  for oats, ND -180  $\mu\text{g kg}^{-1}$  for spring wheat, and ND - 15  $\mu\text{g kg}^{-1}$  for barley (few samples).

Results of CEEREAL on T-2 levels in raw oats, analysed in 2005-2008 in four EU countries (UK, Ireland, Germany and Finland), have been presented (Pettersson, 2008; Pettersson, 2009). Sampling was based on EU regulation 401/2006 and included 400 samples per year. Based on 138 oat samples, the mean T-2 level was 32  $\mu\text{g kg}^{-1}$ , the median was 10  $\mu\text{g kg}^{-1}$ , and the maximum value was 269  $\mu\text{g kg}^{-1}$ .

In Italy, 77 wheat samples harvested in 2009 were analysed for T-2. In all the 27 soft wheat samples, T-2 was below the LOD (1  $\mu\text{g kg}^{-1}$ ), and out of the 50 durum wheat samples, 14 samples were positive for T-2, detected only in traces (Battilani and Pietri, unpublished data).

Field monitoring data (2006-2008) from the Veneto region in Italy showed that T-2 was present in maize and wheat, but the occurrence and level of contamination are low (GLM, 2009). In 2006, 26% of the maize samples were positive (above the LOQ of 1.0  $\mu\text{g kg}^{-1}$ ). In 2007, 21% of the maize samples contained T-2 above the LOQ with a mean of 6.7  $\mu\text{g kg}^{-1}$  (maximum level 18.0  $\mu\text{g kg}^{-1}$ ). In 2008, the T-2 incidence was 23% with a mean of 5.5  $\mu\text{g kg}^{-1}$  (maximum level 8.3  $\mu\text{g kg}^{-1}$ ). In wheat, the T-2 incidence was 8% in 2007 and 29% in 2008. Mean and maximum values were both 1.4  $\mu\text{g kg}^{-1}$  in 2007, and 2.4  $\mu\text{g kg}^{-1}$  and 4.9  $\mu\text{g kg}^{-1}$ , respectively, in 2008.

Data from a field survey held in the Netherlands in 2009 showed that in winter wheat T-2 occurred very infrequently. Out of the 85 samples, 11 were measurable (LOQ  $0.8 \mu\text{g kg}^{-1}$ ) at a very low level, the highest T-2 level found was  $7.0 \mu\text{g kg}^{-1}$  (Van der Fels-Klerx, unpublished data).

AAf, representing the European starch industry, has presented data on T-2 in the raw materials maize and wheat in 2007 and 2008 (preliminarily results for 2008) (aAf, 2009). T-2 was present in 10% of the 290 maize samples in 2007 with a mean level of all samples of  $19 \mu\text{g kg}^{-1}$  and a maximal value of  $230 \mu\text{g kg}^{-1}$ . In 2008, the incidence was 6% (47 samples) with a mean of  $15 \mu\text{g kg}^{-1}$  and a maximum of  $40 \mu\text{g kg}^{-1}$ . In these cases, samples with values below the LOQ (with a varying LOQ per member) were considered to have a T-2 level of half of the LOQ. In wheat, the incidence in the two years was zero percent (all samples  $< \text{LOQ}$ ).

The European Flour Millers have presented results from monitoring on soft wheat and rye from different origins collected by national flour milling associations (EFM, 2009). Data were shown for Germany, Scandinavian countries, France and UK for several years. In the period 2003-2007 (around 700 samples per year), T-2 toxin in soft wheat from Germany was present in (nearly) all samples at a contamination level of less than  $50 \mu\text{g kg}^{-1}$ , in the seasons 2005/06 and 2006/07 some percentages of the samples were found in the range of  $50\text{-}75 \mu\text{g kg}^{-1}$ . Soft wheat in Scandinavian countries contained less than  $50 \mu\text{g T-2 kg}^{-1}$  in all samples in 2005 and 2007. In 2006, 89% of the samples contained less than  $50 \mu\text{g kg}^{-1}$ , about 9% of the samples contained  $10\text{-}50 \mu\text{g kg}^{-1}$ , and about 2% contained more than  $200 \mu\text{g kg}^{-1}$ . Soft wheat from France contained low levels of T-2 in all the study years. The percentage of samples with levels above the LOQ in the different years 2003-2007 was 6%, 2%, 0%, 17% and 0%, respectively. The average T-2 levels were  $21.3 \mu\text{g kg}^{-1}$ ,  $18.9 \mu\text{g kg}^{-1}$ ,  $< \text{LOQ}$ ,  $14 \mu\text{g kg}^{-1}$ , and  $2 \mu\text{g kg}^{-1}$  in the years 2003-2007, respectively. In the UK comparable low levels of T-2 were found in the study years 2003-2007. In rye, results from 2007/08 from Germany/Austria showed that in nearly all samples (n=155) the T-2 level was below  $5 \mu\text{g kg}^{-1}$ , and 2% of the samples had levels in the range of  $5\text{-}10 \mu\text{g kg}^{-1}$ .

Euromalt – representing the EU malting industry – has reported on the T-2 incidence in malting barley harvested in 2004-2007 (Slaiding, 2008; Slaiding, 2009). Data were available from all EU Member States with significant malt production, totalling 100-200 samples per year. The number of samples per country is proportional to malt production. Samples were collected as pairs: a barley sample and a sample of the malt produced from that barley. The T-2 incidence in barley (LOD  $1 \mu\text{g kg}^{-1}$ ) increased from 2004 (26%) to 2005 (78%) -2006 (76%) and sustained in 2007 (85%) and 2008 (86%) (Slaiding, 2009). The yearly mean and maximum concentrations of T-2 in barley varied over the years of 2004-

2008 (Slaiding, 2008). The annual mean was between 4 – 8  $\mu\text{g kg}^{-1}$  and the maximum level was below 100  $\mu\text{g kg}^{-1}$  each year. Euromalt concluded that the occurrence and concentrations of T-2 in malting barley appears to have increased across Europe since 2004.

### 3.1.2 Feed products

In their study, Scudamore and co-workers (Scudamore et al., 2009) found that de-hulling oats resulted in a co-product in which T-2 was concentrated to a level of more than 100  $\mu\text{g kg}^{-1}$  for all, except one, of the 27 samples. Two samples contained T-2 at a concentration of more than 1000  $\mu\text{g kg}^{-1}$  (maximum value 6120  $\mu\text{g kg}^{-1}$ ). European oat by-products collected in the period 2005-2008 (80 samples) showed the mean, median and maximum T-2 levels were 122  $\mu\text{g kg}^{-1}$ , 66  $\mu\text{g kg}^{-1}$  and 595  $\mu\text{g kg}^{-1}$ , respectively (Pettersson, 2008; Pettersson, 2009).

In Lithuania, the incidence and levels of T-2 and HT-2 in Lithuanian cereals intended for animal feed were examined (Garaleviciene et al., 2002). In total 40 cereal samples, including 23 winter wheat, 12 summer barley, 5 oats and 52 samples of mixed feed for swine and poultry, were collected for testing. The cereal samples were collected just after harvest in July - September 1999 from a state factory in Kaunas. Of the 40 cereal samples, T-2 toxin was found in the oat samples, but not in wheat and barley (LOD 50  $\mu\text{g kg}^{-1}$ ). Three of the five oat samples were positive, with the mean of the positive samples being 526  $\mu\text{g kg}^{-1}$  (maximum value 1454  $\mu\text{g kg}^{-1}$ ). From all 52 mixed feed samples, 17% were positive for T-2 with a mean of 598  $\mu\text{g kg}^{-1}$  (maximum value 3852  $\mu\text{g kg}^{-1}$ ). Values in mixed feed for pigs were higher as compared to mixed feed for poultry.

Binder et al. (2007) studied the occurrence of T-2 in raw feed materials (barley, wheat, oats and maize) and finished feed, sampled directly at animal farms and animal feed production sites in a two year period (October 2003 - December 2005) in Europe. The samples were grouped according to the region of origin, i.e., Northern, Central, and Southern Europe. In Northern Europe, T-2 was found in 40% of the samples tested (maximum level 1776  $\mu\text{g kg}^{-1}$ , median 102  $\mu\text{g kg}^{-1}$ ). Samples sourced in Finland had a high incidence and high levels of T-2. Central European samples (comprising 0.67 of all European samples) showed T-2 was detected in 24% of the samples, with a median of 112  $\mu\text{g kg}^{-1}$ . The southern European and Mediterranean region (123 samples) showed T-2 toxin tested positive in 19% of the samples, and levels detected were low (maximum 60  $\mu\text{g kg}^{-1}$ , median 38  $\mu\text{g kg}^{-1}$ ). Grouping all European samples together to commodities showed the following T-2 contamination results: 1 out of 18 maize samples were positive, 18 out of 83 wheat samples were positive (mean 187  $\mu\text{g kg}^{-1}$ ), 1 out of 5 barley samples were positive, 21 out of 26 oat samples were positive (mean 418  $\mu\text{g kg}^{-1}$ ), and 7 out of 54 finished feed samples were positive (mean 219  $\mu\text{g kg}^{-1}$ ) (Binder et al., 2007).

Bouxin (2009) has presented data from the FEFAC (European Feed Manufacturers Federation) collective monitoring plan in UK, France, Italy, Denmark and Belgium. Different types of feed, including all cereals and cereal by-products (of wheat and oats) for feed use were sampled in the period 2004-2008. For barley (for feed use), all samples contained less than 75  $\mu\text{g T-2 kg}^{-1}$  in 2005 and 2007. In 2004 and 2008, nearly all samples contained less than 75  $\mu\text{g T-2 kg}^{-1}$ , the resulting samples (less than 10%) contained between 75-100  $\mu\text{g kg}^{-1}$ . In 2006, 78% of the samples had levels below 75  $\mu\text{g kg}^{-1}$ , 14% between 75-150  $\mu\text{g kg}^{-1}$ , and 8% between 150-300  $\mu\text{g kg}^{-1}$ . In maize for feed use, all samples contained less than 75  $\mu\text{g T-2 kg}^{-1}$  in 2008. In the other years (2004-2007), most of the samples were in this class as well, but between 5-10% of the samples were found in the range of 75-150  $\mu\text{g kg}^{-1}$ . In addition, 4% of the samples had levels between 150-300  $\mu\text{g kg}^{-1}$  in 2006. In oats for feed use, data were available for 2006-2008 showing 14% of the samples had levels between 150-300  $\mu\text{g kg}^{-1}$  in 2007. The remaining samples were at the level of less than 150  $\mu\text{g kg}^{-1}$ , with 42%, 64% and 92% having less than 75  $\mu\text{g T-2 kg}^{-1}$ , and 58%, 22% and 8% contained levels between 75-150  $\mu\text{g T-2 kg}^{-1}$  in the three consecutive years. In wheat, all samples contained less than 75  $\mu\text{g T-2 kg}^{-1}$  in the years 2004-2008, except 14% of the samples with levels between 75-150  $\mu\text{g kg}^{-1}$  in 2006 and 6% between 150-300  $\mu\text{g kg}^{-1}$  in 2008. Thus, T-2 showed very low incidence in triticale and wheat, low incidence in barley and maize (below 300  $\mu\text{g kg}^{-1}$ ), and higher incidence in oats. In oat by-products (oat feed), data from 2006 and 2007 showed – in the two years - about 32% of the samples contained 75-150  $\mu\text{g kg}^{-1}$ , 18% and 35% between 150-300  $\mu\text{g kg}^{-1}$ , and 50 and 33% between 300-600  $\mu\text{g kg}^{-1}$ . In wheat by-products in 2006-2008, nearly all samples contained less than 75  $\mu\text{g kg}^{-1}$ , with less than 10% between 75-150  $\mu\text{g kg}^{-1}$  in 2007 and 2008. Levels of T2 in compound feed for most species were usually below 100  $\mu\text{g kg}^{-1}$  and, except for a few samples of pig feed containing 200-300  $\mu\text{g kg}^{-1}$ , were below 200  $\mu\text{g kg}^{-1}$  for all species. Thus, T-2 had a low incidence in wheat feed, and a rather high incidence in oat feed (Bouxin, 2009).

### 3.1.3 Food products

In Germany, samples (total n=289) of wheat products (n=130), rye products (n=61), and oat products (n=98) were collected from grain-milling factories and 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. Of the 98 oat samples, 17 were of feed quality (not differentiated in the results). All samples were of German origin from the crop years 2005 and 2006 (Gottschalk et al., 2009). The grain kernel samples were fully processed (including cleaning and dehulling steps), suitable for direct consumption. In total 85% of all wheat samples (n=130), 87% of all rye samples (n=61) and 100% of all oats samples (n=98) were contaminated with T-2 (LOD < 0.7

$\mu\text{g kg}^{-1}$ ). Median levels were  $0.11 \mu\text{g kg}^{-1}$ ,  $0.09 \mu\text{g kg}^{-1}$ , and  $2.2 \mu\text{g kg}^{-1}$ , respectively. Highest (maximum) levels were found in wheat bran ( $1.9 \mu\text{g kg}^{-1}$ ), whole rye flour ( $0.8 \mu\text{g kg}^{-1}$ ) and fine oat flakes ( $34 \mu\text{g kg}^{-1}$ ). Two years earlier, Gottschalk and co-workers (Gottschalk et al., 2007) reported results of 70 oat samples (35 from conventional farming, 35 from organic farming) collected at mills and at wholesale stage from the Bavarian market. All samples were of German origin of the production year 2005. They were at least cleaned and de-hulled oats intended for human consumption. Nine samples were oats, 43 oat flakes, eleven oat bran, and seven oat-containing infant food. Separate T-2 levels are only given for the oat flakes ( $n=43$  samples), the highest contaminated product. Contamination of these oat flake samples with T-2 was 100%, with an mean of  $6.4 \mu\text{g kg}^{-1}$  and a maximum value of  $34 \mu\text{g kg}^{-1}$ . The study may have been part of a later publication (Gottschalk et al., 2009) mentioned above. Also in Germany, a food survey comprising 1016 samples from retail products, was held. T-2 incidence in these products was 13% (LOQ  $1 \mu\text{g kg}^{-1}$ ) (Usleber, 2008).

In the four-year study held in UK manufacture of batches of retail products from wheat and maize (186 samples in total) T-2 could not be detected in the samples (LOQ  $10 \mu\text{g kg}^{-1}$ ). In oats (27 samples), T-2 was mostly found in the hulls after their removal from the groats, resulting in T-2 in oats flakes produced from the groats being below the level of  $65 \mu\text{g kg}^{-1}$ . From the 27 samples, 16 oat flake samples did not contain T-2, eight samples contained T-2 levels between  $10-19 \mu\text{g kg}^{-1}$ , two samples between  $20-49 \mu\text{g kg}^{-1}$ , and one samples between  $50-499 \mu\text{g kg}^{-1}$ . Results on T-2 levels in oat flakes collected in the period 2005-2008 by CEEREAL have been presented (Pettersson, 2008; Pettersson, 2009). In total 381 samples were analysed, resulting in a mean level of  $5 \mu\text{g kg}^{-1}$  and a maximum value of  $38 \mu\text{g kg}^{-1}$ .

Biselli and Hummert (2005) reported results from analyses of 685 food samples of European origin, including different food product categories, for the presence of T-2. In nearly 40% of the samples, T-2 could be determined 'semiquantitatively' above a limit of  $0.2 \mu\text{g kg}^{-1}$ . The highest amounts were observed in maize (mean  $0.8 \mu\text{g kg}^{-1}$  and maximum value  $8.4 \mu\text{g kg}^{-1}$ ), oats or oat-based products (mean  $34 \mu\text{g kg}^{-1}$  and maximum value  $266 \mu\text{g kg}^{-1}$ ), respectively.

A SCOOP (Scientific Cooperation) project on the occurrence of *Fusarium* toxins in food matrices in the European Union (see <http://ec.europa.eu/food/fs/scoop/task3210.pdf>) showed that 20% out of in total 3490 samples collected from eight countries were positive for T-2 (Schothorst and van Egmond, 2004). For T-2 in wheat and wheat flour, in total 21% of the 1417 samples were positive, which a LOD ranging between  $2-160 \mu\text{g kg}^{-1}$ . Samples were derived from seven countries including Denmark, Finland, France, Italy, Norway, Portugal and UK. The overall mean (mean of all samples) varied

between 1.7  $\mu\text{g kg}^{-1}$  (UK) and 90  $\mu\text{g kg}^{-1}$  (Denmark). The weighed overall mean was 15  $\mu\text{g kg}^{-1}$  and the weighed mean of the positive samples was 28  $\mu\text{g kg}^{-1}$ . In barley, the total number of results was 502 with 3% of the samples being positive for T-2, with the LOD ranging between 1.7  $\mu\text{g kg}^{-1}$  (UK) - 280  $\mu\text{g kg}^{-1}$  (Italy). The overall mean varied from 0.8  $\mu\text{g kg}^{-1}$  (UK) to 280  $\mu\text{g kg}^{-1}$  (Italy). Four countries (Finland, France, Italy, and UK) provided contributions. In oats, the total number of samples was 464 samples of which 16% was positive, with a LOD ranging between 10  $\mu\text{g kg}^{-1}$  (Finland) and 550  $\mu\text{g kg}^{-1}$  (Austria). The overall mean ranged between 4.2  $\mu\text{g kg}^{-1}$  (Finland) - 68  $\mu\text{g kg}^{-1}$  (Austria). Three countries (Austria, Finland and Norway) provided contributions. For T-2 in rye (and rye flour), in total 62 records from three countries (Denmark, Finland and Norway) were retrieved. Out of these 62 records, 21% was positive, with a LOD ranging from 10  $\mu\text{g kg}^{-1}$  (Finland) to 193  $\mu\text{g kg}^{-1}$  (Denmark). Data from three countries (Austria, France and Italy) showed the incidence of T-2 in corn was 28%, which ranged from LOD 3  $\mu\text{g kg}^{-1}$  (France) to 255  $\mu\text{g kg}^{-1}$  (Austria). The total number of samples was 293.

Data on the T-2 incidence in malt produced from malting barley that was harvested in 2004-2007 has been presented by Slaiding (2008). T-2 was present (LOD 1  $\mu\text{g kg}^{-1}$ ) in only a few percent of the samples in 2004, in about 16% of the samples in 2005-2006, and in 68% of the samples in 2007. The yearly mean and maximum T-2 levels were very low, with the mean being below 3  $\mu\text{g kg}^{-1}$ . Slaiding (2008) concluded that, although the incidence of T-2 in barley seems to have stabilized at around 80% of the harvest samples, the incidence in malt continues to increase over 2004-2007.

European produced beer samples were collected in 2006 and 2007, including 195 and 198 samples from 26 and 27 countries in the two years, respectively (Cantrell, 2008). In 2007, most beers were lager beers (n=149), 10 of the beers were classified as white beers, 11 as dark beers and 28 as special beers. T-2 levels did not vary significantly between the different beer types (LOQ 0.10  $\mu\text{g/liter}$ ). The mean T-2 level was 0.098  $\mu\text{g/liter}$  in 2006 (maximum 0.73  $\mu\text{g/liter}$ ) and 0.053  $\mu\text{g/liter}$  in 2007 (maximum 2.67  $\mu\text{g/liter}$ ).

## 3.2 Occurrence of HT-2 toxin

### 3.2.1 Field and commodities

In the period 2001-2005, the occurrence of HT-2 in wheat, barley and oats in the UK has been examined (Edwards 2009a; Edwards 2009b; Edwards 2009c). In wheat and barley, HT-2 was detected in 31% and 36% of the samples, respectively (LOD 10  $\mu\text{g kg}^{-1}$ ). The concentration was usually low, with mean and median concentrations of all samples at or below 10  $\mu\text{g kg}^{-1}$ . In oats, 92% of the samples were contaminated with HT-2 (LOD 10  $\mu\text{g kg}^{-1}$ ). The concentration was frequently high: the mean and median concentration of all samples were 430  $\mu\text{g kg}^{-1}$  and 151  $\mu\text{g kg}^{-1}$ , respectively, and the maximum level was 7584  $\mu\text{g kg}^{-1}$ .

A four-year study investigating wheat, oats and maize at intake of UK mills (Scudamore et al., 2009) could not detect HT-2 in 48 out of 60 wheat samples (LOD 10  $\mu\text{g kg}^{-1}$ ). Ten of the resulting 12 samples contained HT-2 in the range of 10-19  $\mu\text{g kg}^{-1}$ , and two samples contained between 20-49  $\mu\text{g kg}^{-1}$ . Of the 21 samples taken of oats from UK/Ireland, 14 samples contained 50-499  $\mu\text{g kg}^{-1}$  HT-2 and four samples contained 500-999  $\mu\text{g kg}^{-1}$  HT-2 (maximum value 3570  $\mu\text{g kg}^{-1}$ ). For the six oat samples from Scandinavia, HT-2 levels were in the range of 50-499  $\mu\text{g kg}^{-1}$  for four samples and between 500-999  $\mu\text{g kg}^{-1}$  for two samples (maximum value 730  $\mu\text{g kg}^{-1}$ ). At intake of maize, HT-2 was not found in 22 out of the 56 consignments from France. The levels of HT-2 in the resulting 34 positive samples were: 15 samples in the range of 10-19  $\mu\text{g kg}^{-1}$ , 14 samples in the range of 20-49  $\mu\text{g kg}^{-1}$ , and 5 samples in the range of 50-499  $\mu\text{g kg}^{-1}$  (maximum value 149  $\mu\text{g kg}^{-1}$ ).

In Norway, Langseth and Rundberget (1999) reported HT-2 contamination in cereals grown in the period 1996-1998. Of the 102 barley samples, 22% was positive for HT-2 (LOD 20  $\mu\text{g kg}^{-1}$ ), with a mean of the positive samples of 73  $\mu\text{g kg}^{-1}$  (maximum value 440  $\mu\text{g kg}^{-1}$ ). Of the 178 oat samples, 70% was contaminated, with a mean level of 115  $\mu\text{g kg}^{-1}$  and a maximum level of 880  $\mu\text{g kg}^{-1}$ . Of the 169 wheat samples, 1.2% was contaminated, the mean level and maximum level both being 20  $\mu\text{g kg}^{-1}$ . More recent data on HT-2 in Norwegian cereals have been presented by Brodal et al. (2008). HT-2 levels in oats were between ND (not detected; LOD not specified) – 940  $\mu\text{g kg}^{-1}$  in 2006 and between ND-600  $\mu\text{g kg}^{-1}$  in 2007. In spring wheat, HT-2 could not be detected in both years. In barley, HT-2 levels were between ND– LOQ (level not specified) in 2006 and ND -50  $\mu\text{g kg}^{-1}$  in 2007 (few samples in both years). HT-2 data in Norwegian cereals for over more than a decade up to 2007 have been shown by Eriksen (2008). The mean HT-2 level in oats was relative stable over the period 1998-2004, and increased during the years 2005-2007 up to 200-240  $\mu\text{g kg}^{-1}$ . In some years, very high maximum

levels were found, particularly in 1997 and 2005 (both years nearly up to  $800 \mu\text{g kg}^{-1}$ ) and 2006 ( $1050 \mu\text{g kg}^{-1}$ ). In wheat, both the mean and median HT-2 levels in the period 1998-2007 showed to be relative constant: the mean ranged between  $5 \mu\text{g kg}^{-1}$  (2001) -  $15 \mu\text{g kg}^{-1}$  (1999). In the period 2002 - 2007 the mean HT-2 in wheat was very constant at the level of  $10 \mu\text{g kg}^{-1}$ , with annual maximum values of  $20 \mu\text{g kg}^{-1}$ . It was concluded that levels of HT-2 in oats in Norway may be increasing, and there are lots not suitable for consumption (Eriksen, 2008).

In the study held in 1997 in Poland, HT-2 was found in 24% of the 99 oat samples tested, with a mean level of  $21 \mu\text{g kg}^{-1}$  (range  $10 - 47 \mu\text{g kg}^{-1}$ ) (Perkowski and Basiński, 2002). In a later study held in 2003 in Poland (Perkowski et al., 2007), HT-2 was found in 7 of the 32 wheat samples (LOD  $4 \mu\text{g kg}^{-1}$ ). One of the 12 organic wheat samples was positive versus six out of 20 conventional wheat samples. HT-2 levels in the seven positive samples were low, ranging from  $4 - 66 \mu\text{g kg}^{-1}$ .

Results of CEEREAL of HT-2 levels in oats and products thereof from the harvest years 2005-2008 have been presented (Pettersson, 2008; Pettersson, 2009). Based on 138 samples of raw oats, the mean HT-2 level was  $67 \mu\text{g kg}^{-1}$ , with a median and maximum level of  $15 \mu\text{g kg}^{-1}$  and  $572 \mu\text{g kg}^{-1}$ , respectively.

In Italy, the level of HT-2 toxin has been analysed in 77 wheat samples in 2009. All 27 soft wheat samples had a contamination level below LOD ( $1 \mu\text{g kg}^{-1}$ ). Of the 50 durum wheat samples, 10 samples (20%) were contaminated with HT-2, but only traces could be found (Battilani and Pietri, unpublished data). The regional field monitoring program in Italy, held in the period 2006-2008 (GLM, 2009) showed that, generally, HT-2 levels in maize and wheat from the Veneto region were low. In maize, HT-2 was found in 19%, 39%, and 23% of the samples in the three consecutive years. The mean contamination levels were  $6.4 \mu\text{g kg}^{-1}$  (maximum  $14.2 \mu\text{g kg}^{-1}$ ) in 2007 and  $8.2 \mu\text{g kg}^{-1}$  (maximum  $13.1 \mu\text{g kg}^{-1}$ ) in 2008. In wheat, HT-2 was not found in 2007. In 2008, the toxin was found in 29% of the samples, with a mean of  $3.7 \mu\text{g kg}^{-1}$  and a maximum of  $13.7 \mu\text{g kg}^{-1}$ .

A field survey held among 85 private farmers in the Netherlands in 2009 showed that HT-2 incidence in winter wheat was low. Only, four samples were positive (LOD  $10 \mu\text{g kg}^{-1}$ ) with a maximal level of  $38 \mu\text{g kg}^{-1}$  (Van der Fels-Klerx, unpublished data).

The European Flour Millers (EFM, 2009) presented results for HT-2 in soft wheat and rye from different European countries in the period 2003-2007 (about 700 samples per year). In soft wheat from Germany, the toxin occurred at the level of less than  $50 \mu\text{g kg}^{-1}$  in all samples in the years 2003/2004 and 2004/2005, and in 98% of the samples in 2005/2006 with the other 2% of the samples containing

more than 200  $\mu\text{g kg}^{-1}$ . In 2007/2008, 96% of the samples contained less than 50  $\mu\text{g kg}^{-1}$  HT-2, and the resulting 4% contained varying levels of 1% in each of the other four contamination classes (of 50-75, 75-100, 100-200, > 200  $\mu\text{g kg}^{-1}$ ). In Scandinavian countries, based on about 60 samples of soft wheat collected each year, some higher levels were found. In 2005 85% of the samples contained less than 50  $\mu\text{g HT-2 kg}^{-1}$  and 15% of the samples was in the range of 10-50  $\mu\text{g kg}^{-1}$ . In 2006 72% of the samples contained less than 50  $\mu\text{g HT-2 kg}^{-1}$ , 26% contained between 10-50  $\mu\text{g kg}^{-1}$ , and 2% contained more than 200  $\mu\text{g kg}^{-1}$ . In 2007 all samples contained less than 50  $\mu\text{g HT-2 kg}^{-1}$ . In France, the incidence of HT-2 in soft wheat was 6%, 3%, 0%, and 27% in the years 2003-2006, respectively. Mean values in these years were 21.5  $\mu\text{g kg}^{-1}$ , 18.9  $\mu\text{g kg}^{-1}$ , < LOD (level not specified) and 16.1  $\mu\text{g kg}^{-1}$ , respectively. In the UK, HT-2 contamination of soft wheat showed to be at a comparable low level in the years 2003-2007. Rye from Germany in the period 2007/2008 showed 77% of the 121 samples contained less than 5  $\mu\text{g HT-2 kg}^{-1}$ , about 18% of the samples contained between 5-10  $\mu\text{g kg}^{-1}$ , 3% of the samples was in the range of 10-20  $\mu\text{g kg}^{-1}$ , and 2% of the samples contained between 20-40  $\mu\text{g HT-2 kg}^{-1}$ .

Results of a survey held by AAF (2009) showed HT-2 occurred in 5% of the raw maize samples in 2007 and in 0% of the maize samples in 2008. In 2007, the mean concentration was 9  $\mu\text{g kg}^{-1}$  with a maximum of 25  $\mu\text{g kg}^{-1}$ . In wheat raw material, HT-2 was not found in 2007. In 2008, the incidence was 5% with mean and maximum values of 23  $\mu\text{g kg}^{-1}$  and 30  $\mu\text{g kg}^{-1}$ , respectively.

The incidence of HT-2 in barley (LOD 2  $\mu\text{g kg}^{-1}$ ) harvested in 2004-2008 increased from about 48% in 2004 to between 82-84% in the years 2005-2008 (Slaiding, 2008; Slaiding, 2009). The annual mean and maximum concentrations HT-2 in barley also increased from 2004 to 2007, from a mean level of 7  $\mu\text{g kg}^{-1}$  in 2004, 14  $\mu\text{g kg}^{-1}$  in 2005, 17  $\mu\text{g kg}^{-1}$  in 2006 to 35  $\mu\text{g kg}^{-1}$  in 2007. The maximum level sharply increased in 2007 up to nearly 300  $\mu\text{g kg}^{-1}$ . So, the occurrence and concentrations of HT-2 in barley has increased across Europe since 2004, and stabilized at an incidence of about 80% of the samples. In the related malt samples, the incidence of HT-2 also increased over the years, from about 6% in 2004, 38% in 2005, 42% in 2006, towards 52% in 2007. In the malt, there was a sharp increase in the mean and maximum concentration in the year 2007, with the mean being 7  $\mu\text{g kg}^{-1}$  and the maximum being 90  $\mu\text{g kg}^{-1}$  (Slaiding, 2008).

### 3.2.2 Feed products

In their study, Scudamore and co-workers (2009) found that de-hulling of oats resulted in a co-product in which HT-2 was concentrated to more than 100  $\mu\text{g kg}^{-1}$  for all, except one, of the 27 samples. Fifteen of the samples contained residues of more than 1000  $\mu\text{g kg}^{-1}$ , while HT-2 exceeded 5000  $\mu\text{g kg}^{-1}$  in three samples (maximum 23580  $\mu\text{g kg}^{-1}$ ).

In a study held in Lithuania, HT-2 was found in barley and oats but not in wheat (Garaleviciene et al., 2002). Of the 12 barley samples, 83% were positive with a mean of the positive samples of 19  $\mu\text{g kg}^{-1}$  (maximum value 54  $\mu\text{g kg}^{-1}$ ). All five oat samples collected were positive for HT-2, with a mean of 66  $\mu\text{g kg}^{-1}$  (maximum value 146  $\mu\text{g kg}^{-1}$ ). Of the 52 mixed feed samples, seven samples (13%) showed to be positive for HT-2 with a mean of all positives of 98  $\mu\text{g kg}^{-1}$  (maximum value 126  $\mu\text{g kg}^{-1}$ ). The toxin was found in one of the 25 mixed feed samples for pigs (value 126  $\mu\text{g kg}^{-1}$ ) and in six of the 27 samples of mixed feed for poultry (mean value 70  $\mu\text{g kg}^{-1}$ ).

Based on 80 samples of oat by-products, derived from the harvest years 2005-2008, the mean and median HT-2 level were 196  $\mu\text{g kg}^{-1}$  and 110  $\mu\text{g kg}^{-1}$ , respectively (maximum value 963  $\mu\text{g kg}^{-1}$ ) (Pettersson, 2008; Pettersson, 2009).

Bouxin (2009), presenting results collected by FEFAC, showed that all barley samples were contaminated with HT-2 at the level of less than 75  $\mu\text{g kg}^{-1}$  in the period 2004-2008, except for 18% of the samples containing 50-150  $\mu\text{g HT-2 kg}^{-1}$  in 2006 and 21% of the samples containing 150-300  $\mu\text{g HT-2 kg}^{-1}$  in 2008. No data were available for 2005. In oats for feed use, data were available for 2006-2008. In these three consecutive years, the percentage of samples containing less than 75  $\mu\text{g kg}^{-1}$  HT-2 was 14%, 29% and 84%, respectively. The percentage of samples containing between 50-150  $\mu\text{g HT-2 kg}^{-1}$  were 53%, 20% and 8%, respectively. In 2007 and 2008, 15% and 8% of the samples contained between 150-300  $\mu\text{g kg}^{-1}$ , and in 2006 and 2007, 43% and 36% of the samples contained more than 300  $\mu\text{g kg}^{-1}$ . Thus, the incidence of HT-2 was low in wheat, maize and barley, but the incidence in oats was relatively important (Bouxin, 2009). In oat by-products, HT-2 was found in high levels, namely 200  $\mu\text{g kg}^{-1}$  and higher. In 2006 and 2007, 33% and 28% of the samples contained levels between 200-600  $\mu\text{g kg}^{-1}$ , 17% and 82% of the samples had level ranges of 600-1200  $\mu\text{g kg}^{-1}$ , and in 2006 50% of the samples contained more than 1200  $\mu\text{g kg}^{-1}$ . In wheat by-products (wheat feed) nearly all samples contained less than 50  $\mu\text{g HT-2 kg}^{-1}$  in the years 2006-2008, with up to 9% of the samples ranging between 50-200  $\mu\text{g kg}^{-1}$  in 2006 and 2008. Thus, HT-2 incidence in wheat feed was

low, but rather high in oat feed. Incidence of T-2 and HT-2 in oilseed meals was low (below 50  $\mu\text{g kg}^{-1}$ ) (Bouxin, 2009).

### 3.2.3 Food products

The four-year study held in the UK also investigated HT-2 levels in retail products (Scudamore et al., 2009). From the 146 samples of wheat and maize derived products, only one (snack) was contaminated with HT-2, its level being 12  $\mu\text{g kg}^{-1}$ . In oat flakes, five of the 27 samples were negative for HT-2. Levels of this toxin in the resulting 22 samples were as follows: 9 samples contained 10-19  $\mu\text{g HT-2 kg}^{-1}$ , 12 samples contained 20-49  $\mu\text{g kg}^{-1}$  and one sample contained 50-499  $\mu\text{g kg}^{-1}$  (maximum value 55  $\mu\text{g kg}^{-1}$ ).

In a study held in Germany in products of 2005 and 2006 crops (Gottschalk et al., 2009), the HT-2 contamination rate in all wheat product samples was 94% (n=130), with the highest level of 22  $\mu\text{g kg}^{-1}$  in wheat bran. Of the rye product samples (n=61), 93% was contaminated with HT-2, with the highest level (2.6  $\mu\text{g kg}^{-1}$ ) found in the rye flour. Of all oats samples (n=98), 99% was contaminated with HT-2, with the highest level found in fine oat flakes (51  $\mu\text{g kg}^{-1}$ ). In an earlier report on products from the 2005 crop, Gottschalk et al. (2007) reported separate values for HT-2 in oat flakes. Out of 43 samples, the incidence rate was 100%, with a mean value of 14  $\mu\text{g kg}^{-1}$  and a maximum value of 51  $\mu\text{g kg}^{-1}$ . These results may have been incorporated in the publication mentioned above. A survey comprising 1016 samples from retail products in Germany resulted in an incidence of HT-2 in all samples of 38% (LOQ 1  $\mu\text{g kg}^{-1}$ ) (Usleber, 2008).

Data collected by CEEREAL on HT-2 contamination of oat products from the harvest years 2005-2008 have been presented (Pettersson, 2008; Pettersson, 2009). Based on 381 samples of oat flakes, the mean HT-2 level was 14  $\mu\text{g kg}^{-1}$ , with a median level of 9  $\mu\text{g kg}^{-1}$  and a maximum level of 159  $\mu\text{g kg}^{-1}$ .

A SCOOP (Scientific Cooperation) project on the occurrence of *Fusarium* toxins in the European Union (see <http://ec.europa.eu/food/fs/scoop/task3210.pdf>) showed that 14% of in total 3032 samples provided by six European Union member states was positive for HT-2 toxin (Schothorst and Van Egmond, 2004). Of the 1213 wheat samples, 12% was positive for HT-2 toxin, with the LOD ranging between 3.3-50  $\mu\text{g kg}^{-1}$ . In total 501 barley samples collected by three countries (Finland, France and UK) were analysed, resulting in an HT-2 incidence of 5% (LOD ranging from 1.7-287  $\mu\text{g kg}^{-1}$ ). For oats, in total 464 samples from three countries (Austria, Finland and Norway) were analysed, showing a HT-2 incidence of 41%, with LOD ranging from 10-1150  $\mu\text{g kg}^{-1}$ . Rye and rye flour showed an

incidence of 17%, based on 63 samples delivered by three countries (Denmark, Finland and Norway) and the LOD ranging from 10-70  $\mu\text{g kg}^{-1}$ . Maize showed a comparable incidence of 24%, based on 261 samples from the two countries Austria (LOD 120  $\mu\text{g kg}^{-1}$ ) and France (LOD 3  $\mu\text{g kg}^{-1}$ ).

In an investigation of HT-2 contamination in European beers, the mean HT-2 levels in 2006 and 2007 were 0.30  $\mu\text{g/liter}$  (maximum 2.26  $\mu\text{g/liter}$ ) and 0.14  $\mu\text{g/liter}$  (maximum 0.62  $\mu\text{g/liter}$ ), respectively. In 2007, HT-2 was detected in the majority of the European beers, but was (just like T-2) not linked to a particular type of beer (Cantrell, 2008).

### 3.3 Co-occurrence between toxins

This section presents the co-occurrence between T-2 and HT-2 (section 3.3.1) as well as between T-2/HT-2 and other *Fusarium* mycotoxins (section 3.3.2). In the latter case, results are often related to the combined occurrence of T-2/HT-2.

#### 3.3.1 Co-occurrence T-2 and HT-2

In general, a high relationship is found between T-2 and HT-2. Such a high relationship is expected as the two mycotoxins are produced by the same *Fusarium* species on the same metabolic pathway.

In Norwegian cereals (barley, oats and wheat) from 1996-1998, a strong correlation was found between the amount of T-2 and HT-2, being 0.73 for samples containing T-2 toxin (Langseth and Rundberget, 1999). Gottschalk and co-workers (Gottschalk et al., 2009) reported levels of T-2 and HT-2 to be highly correlated to each other in the three different cereals of wheat, rye and oats (reported R-squares were 0.76 or higher). In UK oats, the relationship between T-2 and HT-2 was highly significant ( $p < 0.001$ ) (Edwards, 2009a). However, in UK barley and wheat, a weak relationship between T-2 and HT-2 was found (Edwards, 2009b; Edwards, 2009c). According to the author, this poor relationship was not expected and contrary to results for oats, which had a much higher incidence and concentration range for the two toxins (Edwards, 2009c).

Reported concentration factors between HT-2 versus T-2 in different cereal grains varied between on average 1.75 and 7 (Langseth and Rundberget, 1999; Scudamore et al., 2007; Barrier-Guillot et al., 2009; Gottschalk et al., 2009). Langseth and Rundberget (1999) reported that for Norwegian cereal

samples (barley, oats and wheat) that contained T-2 toxin, the concentration of this toxin was on average 57% (range 10–200%) of that of HT-2 toxin. In France, field data from 2006 and 2007 showed that the level of HT-2 toxin in cereals (wheat, durum wheat, winter barley, spring barley) was about 2.5 times higher than T-2 toxin (Barrier-Guillot et al., 2009). Gottschalk et al. (2009) found that in wheat, HT-2 was about seven-fold higher than T-2, whereas in oats HT-2 was nearly two times as high as T-2. Contamination levels in rye were too low to make such a comparison properly. Scudamore et al. (2007) reported concentration ratios of HT-2 to T-2 of on average 3.5 (median 3.5; SD 22.8%), as based on 12 samples of raw oats.

In food products the relationship between T-2 and HT-2 seems to be weak or even absent. A two year survey among European beers showed no relationship between the two toxins in each of the two years (Cantrell, 2008). Data from a survey from the German food market showed only a weak correlation between T-2 and HT-2 in highly contaminated samples ( $> 20 \mu\text{g kg}^{-1}$  total toxins,  $n=43$ ) (FF2008).

### 3.3.2 Co-occurrence T-2/HT-2 with other toxins

HT-2 and T-2 seem to have no correlation with the toxins DON and nivalenol (NIV) in the cereal grains wheat, barley and oats. This is consistent with the known taxonomy of *Fusarium* which reports that T-2 and HT-2 are produced by different *Fusarium* species – having different environmental requirements – to those that produce DON (usually *F. graminearum* and *F. culmorum*) (Edwards, 2009a). This emphasises that it is unsound to try and imply the occurrence of T-2 and HT-2 from the occurrence of DON in a sample.

Scudamore et al. (2009) investigated DON levels in 22 samples of wheat bran that were positive for HT-2. They found no significant regression between these two toxins ( $P=0.183$ ,  $R^2=0.041$ ). Based on 12 samples of raw oats received by a commercial oat mill, Scudamore et al. (2007) reported no correlation between HT-2 and DON, and poor relationships between HT-2 and NIV as well as between T-2 and NIV. In UK oats, a simple mutual exclusion between HT-2/T-2 (combined) and DON was found, meaning when one is high, the other is low and vice versa (Edwards, 2009a). Also, both NIV and DON showed signs of mutual exclusion with HT-2 in UK oats (Edwards, 2009b). The same pattern was found in wheat and barley from the UK (Edwards et al., 2009).

In France, data from 26 wheat samples collected in 2005 showed mutual exclusion between DON and T-2/HT-2 (combined). In the Arvalis field surveys in 2006 and 2007, T-2/HT-2 (combined) again showed a strong exclusive effect with DON in wheat and barley (including wheat, durum wheat, winter barley, spring barley). In barley, T-2 and HT-2 levels varied, but DON was generally very low,

whereas in wheat and durum wheat DON levels varied and T-2/HT-2 levels were low (Barrier-Guillot et al., 2009). An EU wide survey, collecting 95 samples of malting barley harvested in 2008, also showed a very poor relationship between T-2/HT-2 versus DON (Slaiding, 2009).

The above mentioned results could not be confirmed by Langseth and Rundberget (1999) who reported a significant co-occurrence of the toxins DON, NIV and HT-2/T-2 in various Norwegian cereals (barley, wheat and oats). The group of samples that was positive for the one toxin contained significantly higher amounts of one or both of the two other toxins as compared to the group that did not contain the toxin, but the correlation was not concentration dependent.

Type A trichothecenes are expected to be related to each other as they are produced via the same metabolic pathway by the same species (Thrane et al., 2004). In a publication on the occurrence of mycotoxins in oats, Scudamore et al. (2007) reported that T-2 triol and neosolaniol, two other trichothecenes structurally closely related to T-2 and HT-2, also occurred and seemed to have a fairly constant relationship with T-2 and HT-2. Their levels in oats, at least, suggest that they occur at about 5% of that of HT-2. In accordance to this, in UK oats T-2 triol and neosolaniol were detected in 41% of samples and always as a low concentration co-contaminant in the presence of a high concentration of the primary contaminants T-2 and HT-2 (Edwards, 2009a). The relationships between HT-2 and all other individual detected type A trichothecenes (T-2, neosolaniol, T-2 triol) were all highly significant ( $p < 0.001$ ). HT-2 was always detected when other type A trichothecenes were present within a sample. In UK wheat, T-2 triol was detected in very few samples and always in a low concentration as a co-contaminant in the presence of a high concentration of HT-2 (Edwards, 2009c).

### **3.4 Influencing factors**

#### **3.4.1 Field**

The best way to control T-2 and HT-2 is at the field level, but the question is how. The EU recommendation on the prevention and reduction of *Fusarium* toxins in cereals (REF) are not applicable for these two toxins, as other *Fusarium* fungi (mainly *F. langsethiae*) with different biology are responsible for these two toxins. At this moment, the time and way of infection of this *Fusarium* species is unknown. Also, field data have shown mutual exclusion of DON with T-2/HT-2 (see section 3.3.2) implying when the one toxin is high the other is low (and vice versa). The consequence is that agronomic factors applied to reduce DON levels in the field may not reduced T-2/HT-2 levels.

#### 3.4.1.1 Year / region effects

Langseth and Rundberget (1999) reported a north south gradient for T-2 and HT-2 levels in Norwegian oats. Significantly higher levels of the toxins were observed in the southern regions, as compared to the mid regions, which was again significantly higher than in the northern region of Norway. In UK wheat, T-2/HT-2 incidence varied between years and region (Edwards, 2009c). A highly significant interaction ( $p < 0.001$ ) between year (5 years, 2001-2005) and region (6 regions: South UK, East UK, Midland UK, North UK, Scotland, Ireland) was found, but no consistent trend across year or region. In barley, highly significant differences in T-2/HT-2 incidence between years ( $p < 0.001$ ) and regions ( $p < 0.001$ ) were found, but there was no significant interaction ( $p = 0.316$ ). However, analysis of the T-2/HT-2 concentrations in the positive samples ( $n=159$ ) indicated a significant interaction between year and region ( $p = 0.004$ ) (Edwards, 2009b). For oats, there was a highly significant interaction ( $p < 0.001$ ) between year (4 years: 2002-2005) and region on the combined T-2/HT-2 concentration, with high concentrations detected in all UK regions (Edwards, 2009a). The effects of regions and years may be related to weather conditions: warm and dry weather seems to increase T-2 and HT-2 contamination.

#### 3.4.1.2 Variety differences

Cereal grain varieties differ in their susceptibility for T-2 and HT-2 contamination. Results from trials with oats during 2005-2007 in Sweden (Pettersson et al., 2008) showed varietal differences in fungal susceptibility and toxin occurrence in the harvested kernels. Edwards et al. (2009) also found varietal differences in T-2/HT-2 levels in oats, with a mean level of about  $280 \mu\text{g kg}^{-1}$  in winter oats and  $80 \mu\text{g kg}^{-1}$  in spring oats. The various winter varieties showed differences in their toxin content, which were presumed to be due to genetics, whereas differences between the spring varieties were small (Edwards et al., 2009).

Barley spring varieties seem to have higher T-2/HT-2 contamination than winter varieties. In field surveys held in 2006 and 2007 in France, five times higher levels of T-2/HT-2 (combined) were found in spring barley varieties (about  $50 \mu\text{g kg}^{-1}$ ) as compared to winter varieties (about  $10 \mu\text{g kg}^{-1}$ ) (Barrier-Guillot et al., 2009). EU wide data from the 2007 barley harvest – collected by Euromalt (Slaiding, 2008) – suggested a small difference between winter barley and spring barley, both in incidence and levels of HT-2. The HT-2 incidence in spring barley was 89% versus 82% in winter barley. The mean concentrations were  $44 \mu\text{g kg}^{-1}$  in spring barley (maximum level of  $205 \mu\text{g kg}^{-1}$ ) versus  $41 \mu\text{g kg}^{-1}$  in winter barley (maximum of  $295 \mu\text{g kg}^{-1}$ ). There was a higher percent of samples

of winter barley in the range of 5-10  $\mu\text{g HT-2 kg}^{-1}$ , and a higher percent of spring varieties in the range of 10-50  $\mu\text{g kg}^{-1}$ . T-2 incidence and concentration was slightly lower in winter varieties as compared to spring varieties. The T-2 incidence was 92% in spring barley and 65% in winter barley. The mean T-2 levels were 9.7  $\mu\text{g kg}^{-1}$  (maximum of 69  $\mu\text{g kg}^{-1}$ ) and 4.1  $\mu\text{g kg}^{-1}$  (maximum of 29  $\mu\text{g kg}^{-1}$ ) in spring barley and winter barley, respectively (Slaiding, 2008). Data of the 2008 barley harvest collected by Euromalt (FF2009) suggested higher levels of T-2/HT-2 (combined) in spring barley as compared to winter barley. Fournier (2009) (Fournier, 2009) presented data on T-2/HT-2 levels in malting barley harvested in the years 2006-2008. First of all, they found an increase of the T-2/HT-2 incidence in malting barley from 2003 to over 90% in 2008. In each of the three years, the T-2/HT-2 level was higher in spring barley as compared to winter barley. In spring and winter barley, the mean levels were 73  $\mu\text{g kg}^{-1}$  and 5  $\mu\text{g kg}^{-1}$  in 2006, 59  $\mu\text{g kg}^{-1}$  and 2  $\mu\text{g kg}^{-1}$  in 2007, and 35  $\mu\text{g kg}^{-1}$  and 12  $\mu\text{g kg}^{-1}$  in 2008, respectively. Maximum levels in spring and winter barley were 708  $\mu\text{g kg}^{-1}$  and 41  $\mu\text{g kg}^{-1}$  in 2007, 489  $\mu\text{g kg}^{-1}$  and 63.5  $\mu\text{g kg}^{-1}$  in 2007, and 146  $\mu\text{g kg}^{-1}$  and 29  $\mu\text{g kg}^{-1}$  in 2008.

The initial differences in T-2 and HT-2 contamination of barley winter and spring varieties disappeared during the steeping process, resulting in comparable levels in the final malt (Slaiding, 2008). Generally, both the T-2 and HT-2 incidences and mean concentrations were somewhat lower in winter malt as compared to spring malt. Incidences in winter malt versus spring malt were 68% and 53% for HT-2, and 59% versus 66% for T-2. The mean HT-2 concentrations were 9.7  $\mu\text{g kg}^{-1}$  in spring malt (maximum level 83  $\mu\text{g kg}^{-1}$ ) versus 4.1  $\mu\text{g kg}^{-1}$  in winter malt (maximum level 14  $\mu\text{g kg}^{-1}$ ). The mean T-2 levels were 2.0 (maximum level 8.7  $\mu\text{g kg}^{-1}$ ) and 0.7  $\mu\text{g kg}^{-1}$  (maximum level 2.8  $\mu\text{g kg}^{-1}$ ) in spring and winter malt, respectively.

#### 3.4.1.3 Sowing date

Field data showed that T-2/HT-2 levels are higher with sowing in spring as compared to autumn sowing. The 2008 field survey held in France (n=292) showed that T-2/HT-2 levels in spring barley were around 10  $\mu\text{g kg}^{-1}$  for sowing in October, as compared to up to 30  $\mu\text{g kg}^{-1}$  for sowing in March-April. Another trial with six cultivars and two sowing dates showed that T-2/HT-2 levels were two times lower with autumn sowing (around 20  $\mu\text{g kg}^{-1}$ ) as compared to sowing in February (over 40  $\mu\text{g kg}^{-1}$ ) (Barrier-Guillot et al., 2009). For spring barley, the sowing date is very important as sowing in spring results in much higher T-2/HT-2 levels as compared to sowing in autumn (Barrier-Guillot et al., 2009; Fournier, 2009).

#### 3.4.1.3 Pre-crop

The crop grown in the previous year may have an effect on T-2/HT-2 contamination. A field survey (n=292) held in 2008 in France showed that T-2/HT-2 in spring barley differed according to the previous crop (Barrier-Guillot et al., 2009). With the small grain cereals barley and wheat as precrop, the combined toxin level was higher – 28  $\mu\text{g kg}^{-1}$  and 20  $\mu\text{g kg}^{-1}$ , respectively - as compared to maize, beet and other precrops (all around 10  $\mu\text{g kg}^{-1}$ ). Also, T-2/HT-2 contents increase with the number of small grain cereals during the two previous years, from zero to two years in a row (Barrier-Guillot et al., 2009). In accordance to this, Hasjlova (2009) showed higher T-2 and HT-2 levels in barley grown after cereals as compared to barley grown after maize. Norwegian data showed that the highest levels of T-2/HT-2 in oats were found with growing oats after oats, with a maximal value up to 3000  $\mu\text{g kg}^{-1}$ . Lower levels were found for growing oats after winter wheat, spring wheat and barley (Klemsdal et al., 2009). In accordance to this, Haslova (2009) showed higher T-2 and HT-2 levels in oats with cereals as a precrop, followed by maize, and then by rape as precrop.

#### 3.4.1.4 Fungicide use

Based on the findings up to date, no or only a small effect of the use of fungicides on the contamination of cereals with T-2 and HT-2 has been found. Fungicide trials in oats grown in Norway – with four different combinations of fungicides, stage of application and doses - showed no effect of fungicide treatment on T-2/HT-2 levels (combined). The four treatments resulted in levels between 249 to 301  $\mu\text{g kg}^{-1}$  in the treated oats as compared to 264  $\mu\text{g kg}^{-1}$  in the untreated oats (Klemsdal et al., 2009). Trials held in 2005 in the UK with different fungicides and locations found high levels of T-2 and HT-2, but low effects of the fungicides (Edwards et al., 2009). In 2006, at most locations a reduction of the two toxins was found after using the fungicide, but the opposite was seen in 2007. In their fungicide trials, Fournier (2009) showed treatment could lead to a decrease of T-2/HT-2 in barley, probably because of an indirect action on the responsible fungi (change in balance between the different populations). They concluded that more studies should be conducted to follow the evolution and population between the other *Fusarium* species (Fournier, 2009).

#### 3.4.1.5 Organic production

Organic cultured cereal grains seem to have lower T-2 and HT-2 contamination as compared to conventional cultured cereals, at least in wheat and oats. In the UK, a significant difference was found between the T-2/HT-2 incidence in organic and conventional wheat samples (Edwards, 2009c). The predicted T-2/HT-2 incidence in organic samples was 20% as compared to 36% in conventional samples. The T-2/HT-2 concentration in positive samples (n=534) also indicated a significant

difference between organic and conventional wheat samples. The predicted T-2/HT-2 concentration of positive samples was  $19 \mu\text{g kg}^{-1}$  in organic wheat as compared to  $23 \mu\text{g kg}^{-1}$  in conventional wheat. It is important to note that the overall incidence and concentration of the two toxins in organic and conventional wheat were low. In oats also a highly significant difference in the T-2/HT-2 concentration between organic and conventional oat samples was found. Predicted means of the T-2/HT-2 concentration were  $50 \mu\text{g kg}^{-1}$  and  $264 \mu\text{g kg}^{-1}$  for organic and conventional oats, respectively. The percentage of organic and conventional samples exceeding  $500 \mu\text{g T-2/HT-2 kg}^{-1}$  was fluctuating. In the period 2002-2005, the annual percentage was 0% – 22% for organic oats and 18% - 50% for conventional oats (Edwards, 2009a). In contrast to wheat and oats, the T-2/HT-2 incidence of barley did not significantly differ between conventional and organic samples. Also, no significant difference in the concentration of the positive samples ( $n = 159$ ) was found (Edwards, 2009b).

In oat food products, including oat flakes, oat bran, oats and infant food, a significant difference in the toxin levels of products originating from organic and conventional agriculture has been found (Gottschalk et al., 2007). Based on an equal number of organic and conventional food products, a significant difference has been calculated for T-2, HT-2, T-2 tetraol and T-2 triol. The conventionally produced oat products (35 samples) had a mean T-2/HT-2 level of  $27 \pm 21 \mu\text{g kg}^{-1}$ , and all organic food samples (35 samples) were contaminated with on average  $7.6 \pm 4.6 \mu\text{g kg}^{-1}$ . For the in total 43 oat flakes samples, the mean T-2/HT-2 contamination was  $31 \mu\text{g kg}^{-1}$  for conventional samples (25 samples) versus  $6.9 \mu\text{g kg}^{-1}$  for the organic samples (18 samples).

### 3.4.2 Processing

Processing cereals starts directly at/after harvest with cleaning and sorting the grain. Further processes depend on the cereal type and final feed/food product. Processing cereals will substantially reduce T-2 and HT-2 contamination in products for human consumption but may increase levels in feed products. In general, the following effects of various processes on T-2/HT-2 are seen:

- Cleaning, sorting and sieving: reducing effect on T-2/HT-2. Small kernels (less than 2.1 mm) – which often are higher in T-2/HT-2 - are removed;
- Flour milling: T-2 and HT-2 toxins are not destroyed but unevenly redistributed between the fractions. The toxins are most attached to the outer hull of the grain and, therefore, found more in bran and germ fractions as well as whole meal flour. Levels are lower in flours and grits.

However, the milling process attaches the toxins as well to the inner grain fractions or the 'whiter' flours;

- Starch production: achieves a reduction of T-2/HT-2 in wheat and maize starch;
- Dehulling oats, removal of husks: reducing effect on T-2/HT-2. The reduction varies depending on the initial concentration in the raw material: it is most significant (70-95%) at high initial levels, but lower at low initial levels. Dehulling results in high T-2/HT-2 levels in the resulting husks and, hence, the pellet by-product, and lower levels in oat flakes;
- Sortex cleaning of dehulled oats: the discoloured fraction is higher in T-2/HT-2;
- Other final processing, such as such as boiling, fermentation, baking, frying and extrusion, have no impact on T-2 and HT-2 contamination (Scudamore, 2008).

Specific effects of oat processing have been studied both at laboratories and in mills in Sweden (Pettersson et al., 2008). Sorting and sieving to remove debris and small kernels markedly reduced T-2 and HT-2 levels in the kernels for further processing as compared to the harvested oat. Removing the shell by dehulling will further reduce the toxin concentrations both at laboratory scale and in mill processing. At initial T-2/HT-2 levels of 200  $\mu\text{g kg}^{-1}$  or higher in the harvested oats, normal cleaning and dehulling during mill processing will reduce these levels by 80-95%, but the reduction is lower at lower initial toxin levels. The levels of T-2 and HT-2 could be further reduced by using a Sortex equipment to remove discoloured kernels from the dehulled kernel fractions. The discoloured kernels had mostly 10 times higher toxin concentrations than the kernels with normal colour.

Comparable findings have been reported by Scudamore et al. (2007), investigating the fate of T-2 and HT-2 in 12 lots of raw oats during processing into finished oat flakes and the pellet co-product. The reduction in the toxin levels from raw oats into the oat flakes showed to be large: the mean reduction was 89.4% (range 75% - 98%) for T-2 and 94.9% (range 91.7% - 99.3%) for HT-2. When the percentage HT-2 reduction is plotted against the initial toxin value in the raw oats, the reduction achieved appears to depend on the initial level of contamination. The reduction was highest in the most highly contaminated samples. The same occurred for T-2 although the toxin concentrations were less than for HT-2. The two toxins were concentrated into the pellet by-product: the increase from the raw oats to the by-product averaged 4.6 (range 1.6 - 10.3) for T-2 and 4.2 (range 1.7 - 7.9) for HT-2. The resulting toxin concentrations in the pellet by-product reached more than 6000  $\mu\text{g kg}^{-1}$  for T-2 and 20000 for HT-2 (Scudamore et al., 2007).

Pettersson (2008, 2009) showed comparable results. Based on 47 trials, the percentage reduction of T-2/HT-2 contamination (combined) by processing oats (mainly dehulling) averaged 75% (SD 23%, median 84%). For HT-2 only, the summarized results from 50 trials showed a mean reduction of 79% (SD 19%, median 85%). The two toxins will increase in the oat by-products; products that are used for animal feeding. Based on 40 trials, T-2/HT-2 levels increased in the by-products by on average 420% (SD 343%, median 340%). For HT-2 only, the results of 48 trials showed a mean increase of 350% (SD 331%, median 280%).

In Finland, a research project on the effects of sorting and dehulling on the trichothecene contents in 75 grain samples from the 2007 harvest, including malting barley (24 samples) and oats (51 samples), has been carried out. Sorting and dehulling decreased T-2 and HT-2 concentrations to 67% of the initial value. Sorting had the greatest effect on the levels of T-2 and HT-2. After dehulling, the toxin levels in all samples were below the LOQ of 25  $\mu\text{g kg}^{-1}$ , starting from their initial median level of 68  $\mu\text{g kg}^{-1}$  (combined) (Hietaniemi et al., 2009).

For 22 out of the 35 wheat samples taken at mill intake, Scudamore et al. (2009) reported separate toxin values for the different wheat fractions (wheat, flour, germ, bran). All wheat flour samples were negative for both T-2 and HT-2 (LOQ 10  $\mu\text{g kg}^{-1}$  for both toxins). T-2 occurred in only two of the intact wheat samples (levels of 10  $\mu\text{g kg}^{-1}$  and 12  $\mu\text{g kg}^{-1}$ ), but 12 of the germ and 16 of the bran contained T-2 levels up to a maximum value of 34  $\mu\text{g kg}^{-1}$  and 77  $\mu\text{g kg}^{-1}$ , respectively. This suggest a concentration factor of two to three in the germ and about six in the bran. Similarly, HT-2 occurred in two intake samples (LOQ 10  $\mu\text{g kg}^{-1}$ ) but nine of the germ and six of the bran were positive up to a maximum of 95  $\mu\text{g kg}^{-1}$  (Scudamore et al., 2009).

In the four-year study held in the UK little information could be obtained on the fate of HT-2 during milling and food processing of wheat and maize (total of 146 samples). Manufacturing retail products from wheat and maize resulted in one snack product in which HT-2 was detected at the level of 12  $\mu\text{g kg}^{-1}$ ; HT-2 was undetected in the other products (see section 3.2.1. for initial levels). In oat flakes, five of the 27 samples were negative for HT-2. Of the resulting 22 samples, nine samples contained between 10-19  $\mu\text{g HT-2 kg}^{-1}$ , 12 samples contained between 20-49  $\mu\text{g HT-2 kg}^{-1}$  and the remaining one sample contained 55  $\mu\text{g kg}^{-1}$  (Scudamore et al., 2009).

In a study focusing on the redistribution of *Fusarium* toxins during commercial dry milling of maize, T-2 and HT-2 showed to concentrate into the bran, germ and germ meal, as well as screenings as compared with their content in raw and cleaned, tempered maize. However, this study only used two

batches of maize and the initial concentration of both T-2 and HT-2 was low (Schollenberger et al., 2008).

In a study on the effects of milling and baking on the fate of trichothecenes, four wheat batches were samples at different processing steps. Initial levels of both T-2 and HT-2 in the wheat were low: T-2 content was below 5  $\mu\text{g kg}^{-1}$  and HT-2 content was below 22  $\mu\text{g kg}^{-1}$ . The two toxins showed to concentrate in the waste fractions, i.e., screenings and outer layers of the wheat bran, obtained during cleaning of wheat (Lancova et al., 2008a).

Producing malt from raw barley grain includes the following processing steps: steeping, germination, and kilning. Steeping is reported to wash out T-2 and HT-2, but the amount lost is variable. Factors that could affect the loss of T-2 and HT-2 during steeping are: amount of water used, temperature of steep water, pH of steep water, number of steeps, duration of steeps, extent of mixing/turbulence during steeping, and thickness of husk. Levels of T-2 and HT-2 in malt are lower than in the starting barley. However, the relationship between the toxins in the starting barley and the corresponding finished malt is not constant. Cantrell (2008) noted that beer brewing had little effect: 65-100% of T-2/HT-2 present in the malt still persists in the beer. Thus, levels of each of the two toxins in the final beer are directly related to contamination of the malt. There are little or no significant losses of T-2/HT-2 in brewing co-products (brewers' grains, brewers' hops, brewers' yeast).

Lancova and co-workers (Lancova et al., 2008b) studied the fate of *Fusarium* toxins, including HT-2, during the malting process. They used two batches of barley grains, which contained HT-2 at the level of less than the LOD of 10  $\mu\text{g kg}^{-1}$  (naturally infected) and 11  $\mu\text{g kg}^{-1}$  (artificially infected). HT-2 levels stayed comparable low during the steeping process. However, during the two consequent steps of germination and kilning, HT-2 levels increased to 17  $\mu\text{g kg}^{-1}$  and 22  $\mu\text{g kg}^{-1}$  for the natural and artificial infected batches, respectively. As a result, HT-2 was 2.1 times higher in the raw barley as compared to the final malt product. Levels of HT-2 in the co-product resulting from removing the rootlets to obtain the final barley malt were very high: 1061  $\mu\text{g kg}^{-1}$  and 493  $\mu\text{g kg}^{-1}$  for the natural and artificially infected batches, respectively. As this waste product is used for animal feed or 'healthy' food supplements, its contamination should be taken into consideration. During the process of brewing beer from malting grits, the level of HT-2 did not change (Lancova et al., 2008b).

Fournier (2008) presented results on studies investigating the fate of T-2 and HT-2 during the production processes of malt based on malting barley. Based on 100% contamination of the starting barley (harvested in 2006) with both T-2 and HT-2, this percentage was zero in the steeped barley, and zero at both mid and end germination. In the final malt, HT-2 was present in about 25% of the samples

and T-2 was present in about 5% of the samples (20% for T-2/HT-2 combined). The barley harvest of 2007 was for 78% contaminated with HT-2 and for 15% contaminated with T-2. The related percentages of these two toxins in the malt were about 35% and 4%, respectively. The overall reduction in both toxins during processing malt from barley was 40%. For both T-2 and HT-2 it was concluded that the elimination in steep out water is large, about 80-100%. The synthesis during germination and kilning varies between 0-50%. There was, however, a large variation in resynthesis during the malting process. Final toxin levels in the malt are always lower as in the starting barley. However, the relationship is not constant (Fournier, 2008; Slaiding 2008; Slaiding, 2009).

The overall correlation between T-2/HT-2 levels in barley and malt have also been studied by using pair wise barley – malt samples, including 80 - 95 paired samples per year, over the period 2005-2008 (Slaiding, 2009). By producing malt, the mean combined toxin levels reduced from 14  $\mu\text{g kg}^{-1}$  to 3  $\mu\text{g kg}^{-1}$  in 2005; from 25  $\mu\text{g kg}^{-1}$  to 4  $\mu\text{g kg}^{-1}$  in 2006; from 42  $\mu\text{g kg}^{-1}$  to 8  $\mu\text{g kg}^{-1}$  in 2007, and from 21  $\mu\text{g kg}^{-1}$  to 5  $\mu\text{g kg}^{-1}$  in 2008. Data of 2008 (95 barley - malt pairs) showed T-2/HT-2 contamination was below the level of detection in most malt samples. The relationship between T-2/HT-2 in malt as compared to barley had an explaining variance of 34%, so this relationship is not very reliable.

### **3.5 Absorption and excretion**

Absorption and excretion have not been reported for all trichothecenes, however, they seem to be rapidly and efficiently absorbed in gastrointestinal tract, whatever the species. After ingestion, T-2 can be detected in pig blood within 30 minutes. Absorption rates vary among trichothecenes and studies between 10% and 50%. T-2 toxin is more rapidly absorbed than DON after its ingestion by most species, with the plasmatic half-life of DON being less than 20 min. The main part of absorbed *Fusarium* toxins shows a rapid elimination within 24 hours after ingestion, followed by a slower excretion of small amounts. However, traces of these toxins or their derivatives can be found in animal products (Cavret and Lecoeur, 2006).

## **4. Conclusions**

In this study, the currently available data on T-2 and HT-2 were collected, compiled and synthesised, including occurrence data, co-occurrence with other mycotoxins and factors influencing the occurrence. From the available data it can be concluded that raw oats can be highly contaminated with T-2 and HT-2 including high incidence and concentrations, followed by barley. Maize occasionally

can be contaminated at a moderate concentration. T-2 and HT-2 contamination of wheat is seen very infrequently and at a low concentration. Feed products that are of major concern include by-products from oat processing (pellets). Food products generally show low incidence and concentration of T-2 and HT-2, however, oat products may contain some T-2 and HT-2. Field factors that influence T-2 and HT-2 include region-year (climate), variety, sowing date, pre-crop, and organic production. Fungicides seem to have no or only a weak effect. Processing cereals will substantially reduce T-2 and HT-2 contamination in most food products due to redistribution over the various fractions. As a consequence, it increases the levels in the by-products, often used for animal feeding. The results of this study are deemed valuable for risk assessments at the European level.

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## Appendix. Structure of trichothecenes

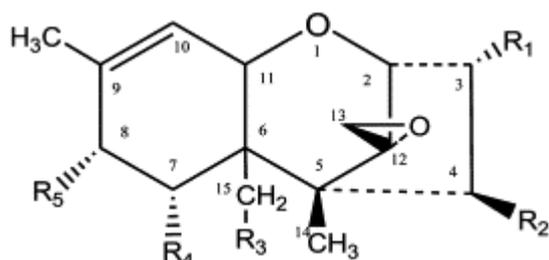


Figure A1. Basic structure of trichothecenes

Table A1. Major trichothecenes and their respective structures<sup>a</sup>

Mycotoxin	Abbreviation	R <sub>1</sub> <sup>b</sup>	R <sub>2</sub> <sup>b</sup>	R <sub>3</sub> <sup>b</sup>	R <sub>4</sub> <sup>b</sup>	R <sub>5</sub> <sup>b</sup>
Type A trichothecenes <sup>c</sup>						
T-2 toxin	T-2	OH	OAc	OAc	H	-OCOCH <sub>2</sub> CH(CH <sub>3</sub> ) <sub>2</sub>
HT-2 toxin	HT-2	OH	OH	OAc	H	-OCOCH <sub>2</sub> CH(CH <sub>3</sub> ) <sub>2</sub>
T-2 triol	T-2 triol	OH	OH	OH	H	-OCOCH <sub>2</sub> CH(CH <sub>3</sub> ) <sub>2</sub>
T-2 tetraol	T-2 tetraol	OH	OH	OH	H	OH
3'-hydroxy T-2	3'-OH T-2	OH	OAc	OAc	H	-OCOCH <sub>2</sub> COH(CH <sub>3</sub> ) <sub>2</sub>
3'-hydroxy HT-2	3'-OH HT-2	OH	OH	OAc	H	-OCOCH <sub>2</sub> COH(CH <sub>3</sub> ) <sub>2</sub>
Neosolaniol	NEO	OH	OAc	OAc	H	OH
Verrucarol	VER	H	OH	OH	H	H
Scirpentriol	SCP	OH	OH	OH	H	H
Diacetoxyscirpenol	DAS	OH	OAc	OAc	H	H
15-monoacetoxyscirpenol	MAS	OH	OH	OAc	H	H

Mycotoxin	Abbreviation	R <sub>1</sub> <sup>b</sup>	R <sub>2</sub> <sup>b</sup>	R <sub>3</sub> <sup>b</sup>	R <sub>4</sub> <sup>b</sup>	R <sub>5</sub> <sup>b</sup>
Type B trichothecenes <sup>c</sup>						
Deoxynivalenol	DON	OH	H	OH	OH	=O
3-acetyldeoxynivalenol	3-ADON	OAc	H	OH	OH	=O
15-acetyldeoxynivalenol	15-ADON	OH	H	OAc	OH	=O
Nivalenol	NIV	OH	OH	OH	OH	=O
Fusarenon-X	FUS	OH	OAc	OH	OH	=O

(a): Derived from He et al. (2009)

(b): "R" stands for "functional group". The positions of these functional groups are referred to in Figure 1.

(c): The type A trichothecenes do not contain a carbonyl function group at C-8 whereas the type B trichothecenes do.

**Table 1. Occurrence of T-2 in Europe**

Origin	Year	Commodity	N of samples	LOQ (µg/kg)	Samples >LOQ (%)	Mean	Median	Min	Max	Reference
UK	2001-2005	Wheat	1624	10	16	<10 <sup>b</sup>	<10		52	Edwards (2009c)
UK	2002-2005	Barley	446	10	12	<10 <sup>b</sup>	<10		39	Edwards (2009b)
UK	2002-2005	Oats	458	10	84	140 <sup>b</sup>	58		2406	Edwards (2009a)
UK	2004-2007	Wheat	60	10	5			<10	13	Scudamore et al. (2009)
UK	2004-2007	Oats	27, of which 6 from Scandinavia	10	100	219		25	1610	Scudamore et al. (2009)
France	2004-2007	Maize	56	10	39			<10	107	Scudamore et al. (2009)
UK	2004-2007	Oat flakes	27	10		21		<10	65	Scudamore et al. (2009)
UK	2004-2007	Oat pellets	27	10	100	921		71	6120	Scudamore et al. (2009)
CEEREAL <sup>a</sup>	2005-2008	Oats	138			32	10		269	Pettersson (2008, 2009)
CEEREAL <sup>a</sup>	2005-2008	Oat flakes	381			5	3		38	Pettersson (2008, 2009)
CEEREAL <sup>a</sup>	2005-2008	Oat by- product	80			122	66		595	Pettersson (2008, 2009)
The Netherlands	2009	Wheat	85	0.8	13				7.0	Van der Fels-Klerx, pers. comm.
Poland	1997	Oats	99	10	15	60		<10	703	Perkowski and Basiński (2002)
Poland	2003	Wheat	32	1.0	0					Perkowski et al. (2007)
EU countries	2004-2007	Spring barley	53	1.0	92	9.7			69	Slaiding (2008)
EU countries	2004-2007	Winter barley	17	1.0	65	4.1			29	Slaiding (2008)
EU countries	2004-2007	Spring malt	53	1.0	66	2.0			8.7	Slaiding (2008)
EU countries	2004-2007	Winter malt	17	1.0	59	0.7			2.8	Slaiding (2008)

Europe	2006	Beers	198	0.10 <sup>c</sup>		0.098 <sup>c</sup>		0.73 <sup>c</sup>		Cantrell (2008)
Europe	2007	Beers	195			0.053 <sup>c</sup>		2.67 <sup>c</sup>		Cantrell (2008)
Italy	2007	Maize	20	1	21	6.7		5.6	18.0	GLM (2009)
Italy	2008	Maize	20	1	23	5.5		4.3	8.3	GLM (2009)
Italy	2007	Wheat	20	1	8	1.4		1.4	1.4	GLM (2009)
Italy	2008	Wheat	20	1	29	2.4		1.1	4.9	GLM (2009)
EU countries	2003	Food products	3490	20						SCOOP 2003
Europe	2007	Maize	290	10		19 <sup>b</sup>		13	230	aAf (2009)
Europe	2008	Maize	47	6		15 <sup>b</sup>		13	40	aAf (2009)
Europe	2007	Wheat	25	0				13	30	aAf (2009)
Europe	2008	Wheat	29	0				13	30	aAf (2009)
Sweden	2005	Oats	41	29		59	14	699		H. Pettersson, pers. comm..
Sweden	2006	Oats	71	67		190	158	700		H. Pettersson, pers. comm..
Sweden	2007	Oats	58	53		102	58	1170		H. Pettersson, pers. comm..
Sweden	2008	Oats	70	52		29	18	174		H. Pettersson, pers. comm..
Norway	1996-1998	Barley	102	20	5	85		<20	220	Langseth and Rundberget (1999)
Norway	1996-1998	Oats	178	20	30	60		<20	380	Langseth and Rundberget (1999)
Norway	1996-1998	Wheat	169	20	0.6	20		<20	20	Langseth and Rundberget (1999)
Germany	2005-2006	Wheat products	130	<1	85	0.21	009	1.9		Gottschalk et al. (2009)
Germany	2005-2006	Rye products	61	<1	87	0.13	0.11	0.8		Gottschalk et al. (2009)
Germany	2005-2006	Oat products	98	<1	100	4.2	2.2	34		Gottschalk et al. (2009)
Lithuania	1999	Oats	5	50	60	526		1454		Garaleviciene et al. (2002)
Lithuania	1999	Mixed feed swine,	52	50	17	598		3852		Garaleviciene et al. (2002)

		poultry					
Lithuania	1999	Wheat	23	50	0		Garaleviciene et al. (2002)
Lithuania	1999	Barley	12	50	0		Garaleviciene et al. (2002)
Europe		Oat food products		0.2	34	266	Biselli and Hummert (2005)
Europe		Maize food products			0.8	8.4	Biselli and Hummert (2005)

(a): CEEREAL data including the countries UK, Ireland, Finland, Germany

(b): refers to all samples

(c): value expressed in µg/liter

**Table 2. Occurrence of HT-2 in Europe**

Origin	Year	Commodity	N of samples	LOQ (µg/kg)	Samples >LOQ (%)	Mean	Median	Min	Max	Reference
UK	2001-2005	Wheat	1624	10	31	<10 <sup>b</sup>	<10		193	Edwards (2009c)
UK	2002-2005	Barley	446	10	36	10 <sup>b</sup>	<10		105	Edwards (2009b)
UK	2002-2005	Oats	458	10	92	430 <sup>b</sup>	151		7584	Edwards (2009a)
UK	2004-2007	Wheat	60	10	20			<10	28	Scudamore et al. (2009)
UK	2004-2007	Oats	21	10	100			>50	3570	Scudamore et al. (2009)
France	2004-2007	Maize	56	10	61			<10	149	Scudamore et al. (2009)
UK	2004-2007	Oat flakes	27	10	81.5	25		<10	55	Scudamore et al. (2009)
UK	2004-2007	Oat pellets	27	10	100	2363		306	2358 0	Scudamore et al. (2009)
CEEREAL <sup>a</sup>	2005-2008	Oats	138			67 <sup>b</sup>	15 <sup>b</sup>		572	Pettersson (2008, 2009)
CEEREAL <sup>a</sup>	2005-2008	Oat flakes	381			14 <sup>b</sup>	9 <sup>b</sup>		159	Pettersson (2008, 2009)
CEEREAL <sup>a</sup>	2005-2008	Oat by-product	80			196 <sup>b</sup>	110 <sup>b</sup>		963	Pettersson (2008, 2009)
The Netherlands	2009	Wheat	85	0.8	5				38	Van der Fels-Klerx, pers. comm.
Poland	1997	Oats	99	10	24	21		<10	47	Perkowski and Basiński (2002)
Poland	2003	Wheat	32	4.0	2	23		<4	66	Perkowski et al. (2007)
EU countries	2004-2007	Spring barley	53		89	44			205	Slaiding (2008)
EU countries	2004-2007	Winter barley	17		82	41			295	Slaiding (2008)
EU countries	2004-2007	Spring malt	53		68	9.7			83	Slaiding (2008)
EU countries	2004-2007	Winter malt	17		59	4.1			14	Slaiding (2008)

Europe	2006	Beers	198	0.10 <sup>c</sup>		0.030 <sup>c</sup>		2.26 <sup>c</sup>	Cantrell (2008)
Europe	2007	Beers	195			0.14 <sup>c</sup>		0.62 <sup>c</sup>	Cantrell (2008)
Italy	2007	Maize	20	0.5	39	6.4	5.1	14.2	GLM (2009)
Italy	2008	Maize	20	0.5	23	8.2	6.8	13.1	GLM (2009)
Italy	2007	Wheat	20	0.5	0	-	-	-	GLM (2009)
Italy	2008	Wheat	20	0.5	29	3.7	0.9	13.7	GLM (2009)
Europe	2007	Maize	20	5	5	9 <sup>b</sup>	5	25	aAf (2009)
Europe	2008	Maize	19	5	0	14 <sup>b</sup>	5	15	aAf (2009)
Europe	2007	Wheat	23	5	0	21 <sup>b</sup>	5	30	aAf (2009)
Europe	2008	Wheat	22	5	5	23 <sup>b</sup>	5	30	aAf (2009)
Sweden	2005	Oats	41		31	195	76	904	H. Pettersson, pers. comm..
Sweden	2006	Oats	71		68	275	214	952	H. Pettersson, pers. comm..
Sweden	2007	Oats	58		56	142	99	555	H. Pettersson, pers. comm..
Sweden	2008	Oats	70		60	31	12	319	H. Pettersson, pers. comm..
Norway	1996-1998	Barley	102	20	22	73	<20	440	Langseth and Rundberget (1999)
Norway	1996-1998	Oats	178	20	70	115		800	Langseth and Rundberget (1999)
Norway	1996-1998	Wheat	169	20	1.2	20		20	Langseth and Rundberget (1999)
Lithuania	1999	Barley	12	10	83	19		54	Garaleviciene et al. (2002)
Lithuania	1999	Oats	5	10	100	66		146	Garaleviciene et al. (2002)
Lithuania	1999	Mixed feeds	52	10	13	98		126	Garaleviciene et al. (2002)
Lithuania	1999	Wheat	23	10	0				Garaleviciene et al. (2002)
Germany	2005-2006	Wheat food products	130	1.1	94	1.6	0.84	22	Gottschalk et al. (2009)
Germany	2005-2006	Rye foods	61	1.1	93	0.56	0.42	2.6	Gottschalk et al. (2009)
Germany	2005-2006	Oat foods	98	1.1	99	10	6.0	51	Gottschalk et al. (2009)

Germany	Food market	1016	1.0	38	Usleber (2008)
Europe	Food products	3032		14	SCOOP (2003)

(a): CEEREAL data including the countries UK, Ireland, Finland, Germany

(b): refers to all samples

(c): value expressed in µg/liter