Food Safety
AUTHORITY OF IRELAND

CHEMICAL

MONITORING & SURVEILLANCE SERIES

Survey of Mycotoxins in Irish Grain Samples 2012

FEBRUARY 2015

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SUMMARY

Mycotoxins are natural chemical substances with toxic effects on humans that are produced by fungi (moulds) growing as contaminants on some food crops (in field and in storage), including certain cereals, nuts and fruit. They include aflatoxins, ochratoxin A, fumonisins, and trichothecenes. Maximum levels (MLs) for the major mycotoxins in a range of food crops have been set by Commission Regulation 1881/2006/EC (1) as amended, which sets maximum levels for chemical contaminants in foodstuffs and Directive 2002/32/EC and Recommendation 2006/576/EC, which set the ML for chemical contaminant in feedstuff. These MLs are set at a very low level for the particular food/feed and mycotoxins in question following the ALARA principle, i.e. as low as reasonably achievable, in order to ensure that adverse effects on consumers' health are minimised.

For certain other mycotoxins, MLs are yet to be adopted but are under consideration. In order to ensure that established MLs are not exceeded, or to gather information on the occurrence of other mycotoxins for which MLs are under consideration, routine surveillance of food and feed is carried out, involving the taking of samples of potentially contaminated produce, followed by laboratory analysis to determine the levels of mycotoxins in the food/feed.

The Food Safety Authority of Ireland (FSAI) in collaboration with Public Analyst Laboratory in Dublin and the Irish Grain and Feed Association (IGFA), carried out checks on the levels of the above mentioned mycotoxins in a variety of cereal products (oats, barley, wheat and maize) grown in Ireland in 2012. The results of these checks show that the levels found in the Irish-produced cereals which were sampled are generally low, with occasional instances of contamination detected, although these are considered to present little risk to the health of the Irish consumer.

INTRODUCTION

Mycotoxins are natural chemicals produced as secondary metabolites by certain fungi which occur as contaminants of some food crops, either in the field or during post-harvest storage. Cereals, nuts, fruits and foods derived from these crops are the most likely to contain mycotoxins. As the name suggests, mycotoxins are toxic to humans and animals, and consumption of food containing high levels of these contaminants may cause illness.

Three types (genera) of fungi are considered the major producers of mycotoxins – *Fusarium*, *Pencillium* and *Aspergillus*. Within each type of fungus, particular species may be mycotoxigenic (2).

It is estimated that 25% of the world's food crops are affected by mycotoxins each year with substantial impact of *Fusarium* species on food contamination. Fungal and especially *Fusarium* species infection of cereals cause three undesired effects:

- It reduces grain yield and quality
- Generates economic losses in livestock feed with contaminated cereal by reducing animal production and
- Can potentially carry over to food where they may also result in acute or chronic toxicity to humans (3)

The impact of mycotoxins in the food chain is an important issue worldwide. In general, consumers perceive less risk from mycotoxins than from other food-related threats such as pesticides, heavy metals or microbial agents. This is due to the fact that, at least in developed countries, mycotoxins rarely cause acute intoxication outbreaks or health emergencies (4).

Nevertheless, good agricultural production, storage and processing practices cannot completely prevent contamination and small quantities of mycotoxins may be present in food and feed. The presence of mycotoxins above certain levels in feed and food is undesirable as they can elicit a number of adverse effects on health, both in humans and animals.

Mycotoxins can affect the immune system, nervous system, liver, kidneys and blood, and some mycotoxins are known to be carcinogenic. The toxicity of mycotoxins varies greatly and effects may be both acute (after a single high dose exposure) and chronic (after repeated low dose exposure). The most important mycotoxins in terms of effects on health are the aflatoxins, ochratoxin A, patulin and the *Fusarium* toxins (2).

Five mycotoxins are considered to be economically and toxicologically important worldwide: aflatoxin (AF), ochratoxin A (OTA), deoxynivalenol (DON) and derivatives, zearalenone (ZON) and derivatives, and fumonisins (FB_1, FB_2). DON, ZON, and fumonisins are produced by various *Fusarium* species.

Fusarium Toxins

A range of *Fusarium* fungi, which are common soil fungi, produce a number of different mycotoxins of the class 'trichothecenes' (T-2 toxin, HT-2 toxin, DON, nivalenol) and some other toxins (ZON and fumonisins). The *Fusarium* fungi are probably the most prevalent toxin-producing fungi of the northern temperature regions of America, Europe (including Ireland) and Asia (5).

Various factors can affect the formation of *Fusarium* toxins including the amount of rainfall and moisture during the growing season and at harvest. As with most mycotoxins, *Fusarium* toxins are chemically stable, can survive food processing and may pose a potential risk to human health as well as livestock. Trichothecenes can be acutely toxic to humans at high levels causing symptoms including sickness, vomiting, diarrhoea and in very extreme cases, even death (6).

Although the *Fusarium* trichothecenes vary greatly in their toxicity, they are acutely toxic, T-2 toxin being probably the most toxic and DON being amongst the least toxic. On the other hand, DON is the most common trichothecenes in food and feed. Nivalenol (NIV), T-2 toxin and HT-2 toxins occur to lesser extents (4).

Chronic effects in animals have been reported to include immunosuppression, reduced uptake and teratogenicity (congenital abnormality of the foetus). The toxicity and potential impact on human health of several of the *Fusarium* toxins had been considered by the European Food Safety Authority (EFSA) and previously by the

European Commission's Scientific Committee on Food (SCF), and as a result, tolerable daily intakes (TDIs) have been set for each of the *Fusarium* toxins (2).

ZON is an oestrogenic mycotoxin that often occurs with DON on wheat and on maize. It has relatively low overall toxicity but it has been shown to have uterotrophic (anti-reproductive) effects in pigs. The effects of this mycotoxin in humans are not clearly established.

Fumonisins may have neurotoxic effects in some animals, and carcinogenicity in humans has been suggested but not proven.

Aflatoxins

Aflatoxins were first identified in the early sixties as the cause of a mysterious outbreak in England called "Turkey X disease". Aflatoxins are naturally occurring mycotoxins that are produced by *Aspergillus flavus* and *Aspergillus parasiticus*, species of fungi. The aflatoxins are considered to be the most toxic and carcinogenic of the mycotoxins with aflatoxins B_1, B_2, G_1 and G_2 being the principal aflatoxins of concern.

Long-term low level exposure to aflatoxins has been associated with liver diseases such as cancer, cirrhosis, hepatitis and jaundice in humans and animals and they are regarded both as genotoxic (DNA-damaging), carcinogenic and as immunosuppressant. OTA also has immunosuppressant, teratogenic (reproductive) and carcinogenic effects, and a clear connection has been shown between nephropathy (kidney disease) and exposure to OTA in humans and animals (2).

Other *Penicillium* mycotoxins such as penicillic acid and citrinin have been found to enhance the toxic effect (synergism) of OTA on liver and kidney carcinogenesis in animals. Patulin is a potent protein synthesis inhibitor and is also regarded as genotoxic (2). In animal toxicity studies, the effects observed include reduced weight gain, impaired kidney function and intestinal effects. Citreoviridin is a neurotoxin in animals, resulting in paralysis and muscular atrophy.

As regards aflatoxins, the SCF expressed in its opinion of 23 September 1994 that aflatoxins were genotoxic carcinogens. Based on that opinion, it was considered appropriate to limit the total aflatoxin content of food (sum of aflatoxins B_1, B_2, $G_1$and G_2) as well as the aflatoxin B_1 content alone, aflatoxin B_1 being by far the most toxic compound (1). Re-evaluations of aflatoxins have also been carried out by EFSA in recent years.

STUDY OUTLINE

In order to ensure that the established maximum limits as laid down in Regulation 1881/2006/EC, Directive 2002/32/EC and Recommendation 2006/576/EC are not exceeded and in order to collect information on occurrence of mycotoxins for which maximum levels have not yet been adopted, routine surveillance of food and feed must be carried out. This involves taking samples of potentially contaminated produce, followed by laboratory analysis to determine the levels of different toxins in these samples.

For the purposes of this report, the Food Safety Authority of Ireland (FSAI) in collaboration with the Public Analyst's Laboratory, Dublin and the Irish Grain and Feed Association, carried out checks on levels of aflatoxins and *Fusarium* toxins in the food chain in a variety of randomly sampled cereals from the 2012 harvest (oats, barley, wheat, rye and maize). The samples in this study were taken as non-statutory surveillance samples, thus no enforcement action was taken. However, all potentially non-compliant samples were followed up with the suppliers.

Levels of mycotoxins can vary significantly year to year due to different factors, such as weather conditions, presence of insects, storage conditions, crop type, etc (4). The exceptionally wet weather of summer 2012 may have resulted in increased growth of fungus in crops, leading to higher levels of mycotoxins and higher rates of non-compliance with regulatory limits.

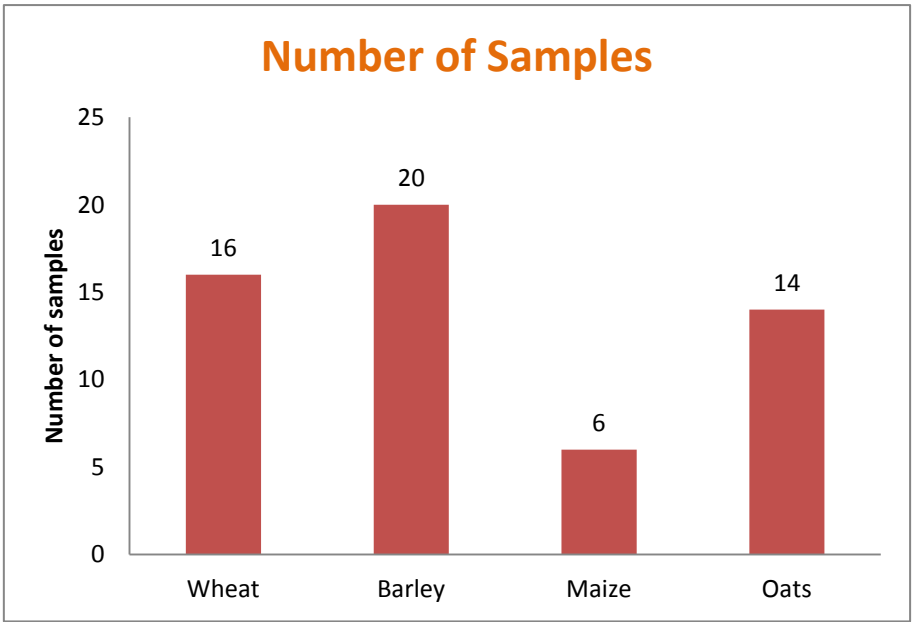
The aim of this survey was to provide a snapshot on the levels of certain mycotoxins in Irish produced cereals from the 2012 harvest.

SAMPLING

Unprocessed cereals were analysed in this survey in order to get a true indication of the underlying levels of the different toxins at source, as toxin levels can be significantly affected by processing.

Furthermore, maximum limits are defined for unprocessed cereals in EU legislation (Commission Regulation (EC) No 1881/2006). The number of samples taken of each type of cereal is shown in Figure 1 below.

Figure 1



Sampling Protocol

By agreement between the parties, sampling of the unprocessed cereals for this survey did not follow the European Union Regulations for this activity (Regulation (EC) No 401/2006 as amended), which requires a large sample size to be taken, i.e. 10 kg, when sampling large lots of cereals. Instead, only 0.5–1 kg of each cereal was submitted to the laboratory, making a total of 56 samples. This reduced the burden to producers that were voluntarily submitting the samples of cereals, while at the same time providing a reliable picture of the levels of mycotoxin contamination of 2012 harvest.

Preparation of the Samples

All the samples were ground to a fine powder using a Grindomix GM300 Knife Mill and stored at ambient temperature in the laboratory prior to analysis.

ANALYTICAL METHODOLOGY

The samples were analysed for the following mycotoxins:

- Aflatoxin B_1, B_2, G_1, G_2 and Total Aflatoxins
- Ochratoxin A
- Zearalenone
- Fumonisin B_1, B_2, B_3 and Total Fumonisins
- Deoxynivalenol
- Nivalenol
- T-2 and HT-2 toxins and sum of T-2 and HT-2 toxins

The analysis was performed according to the following internal laboratory Standard Operating Procedures (SOPs):

- **Aflatoxins–SOP PALC 0031** (7)

  Aflatoxins were extracted from a test portion of the prepared sample with methanol. They were separated from interfering compounds by passing the solution down a column containing monoclonal antibodies specific to aflatoxins. After removal of interfering substances, the aflatoxins were eluted and determined by comparison with aflatoxin standards, using high performance liquid chromatography with post column bromination and fluorescence detection

- **Ochratoxin A–SOP PALC 0018** (8)

  For solid samples, OTA was extracted from a test portion of the prepared sample with dilute sodium bicarbonate solution and the resulting suspension was then clarified. The test solution was diluted with phosphate-buffered saline solution, if specified, and passed through an immunoaffinity column. After removal of interfering compounds, the OTA was eluted and determined by high performance liquid chromatography with fluorescence detection

- **Zearalenone–SOP PALC 0022** (9)

  Samples of cereals (incl. cereal-based baby foods) were homogenised and then a portion was blended in an acetonitrile: water (UPW) mixture to extract the zearalenone. The resulting suspension was clarified. The test solution was diluted with phosphate-buffered saline (PBS), pH adjusted as appropriate, and passed through an immunoaffinity column. After removal of interfering compounds by washing, the retained zearalenone was eluted and determined by high performance liquid chromatography with fluorescence detection

- **Fumonisins–SOP PALC 0076** (10)

  Fumonisins were extracted from cereal matrices with a methanol/acetonitrile/UPW mixture and separated from interfering substances by passing through a column containing monoclonal antibodies specific to fumonisins. The isolated fumonisins were eluted and analysed by HPLC with pre-column derivatisation and fluorescence detection

- **Deoxynivalenol–SOP PALC 0081** (11)

  DON was extracted from all matrices with UPW and separated from interfering substances by passing through a column containing monoclonal antibodies specific for DON. The isolated DON was then eluted and analysed by HPLC and UV detection

- **Nivalenol–SOP PALC 0133** (12)

  NIV is extracted from cereals with UPW and separated from interfering substances by passing through a column containing monoclonal antibodies specific for NIV. The isolated NIV is eluted and analysed by UPLC with UV detection

- **T-2 and HT-2 toxins–SOP PALC 0074** (13)

  The sample of cereals was suspended in water. A portion of ethyl acetate was added and the suspension was agitated. Sodium sulphate was then added to assist phase separation and the solid material was separated by centrifugation. The organic phase was separated and stable isotope-labelled analogues were added to the solution. After mixing well, the liquid was evaporated to dryness and the residue was reconstituted by the addition of a quantity of mobile phase and an equal volume of water. The T-2 and HT-2 content was determined by UPLC with MS/MS detection. The laboratory is accredited by the Irish National Accreditation Board (INAB) to ISO 17025:2005 (Registration No. 99T)

Non-conforming samples

Samples that did not meet the legislative limits for any single parameter or more than one parameter, underwent repeat analysis in accordance with the laboratory's procedures to confirm the results before reporting.

Non-conforming samples were reported to the FSAI as soon as the laboratory became aware of any results that breached legislative limits so that action could be taken in a timely manner, if deemed necessary.

RESULTS

The following section provides an overview of the number of cereal samples exceeding the levels set out in legislation for the mycotoxins analysed. The results were assessed against the legislative limits for food as these are stricter than the legislative limits set down for feed and the proposed use of the cereals had not been determined at the time of sampling. The regulatory levels used for the analysis correspond to the ones set out for the category of unprocessed cereals, which are cereals that have not undergone any physical or thermal treatment other than drying, cleaning and sorting.

The analytical results for each sample and each analyte were assessed against legislative limits, where available.

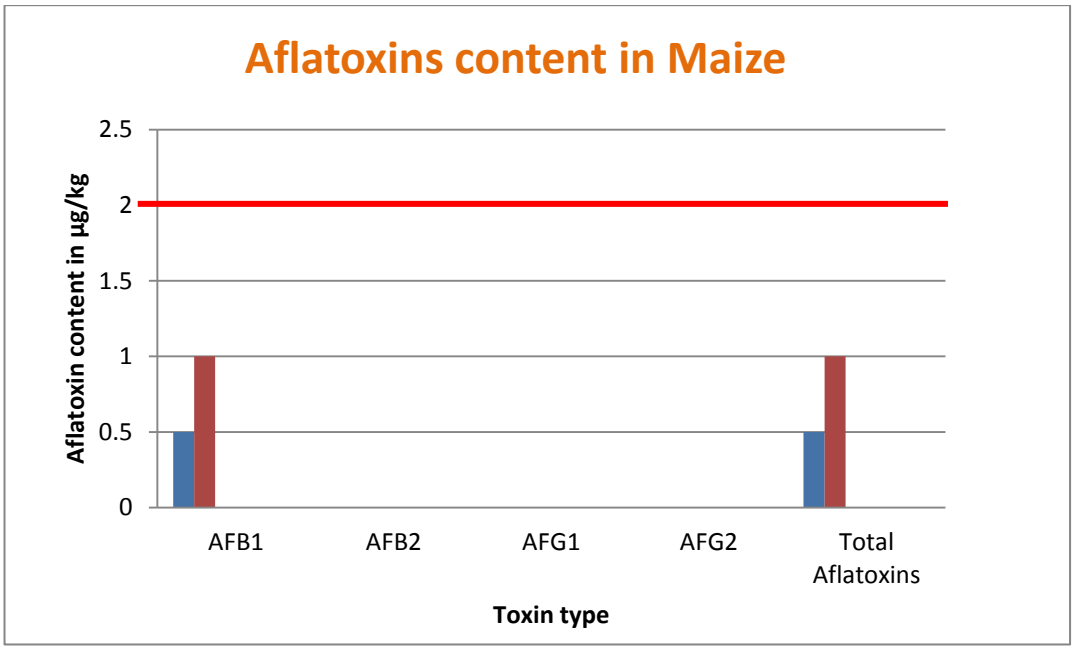
- For aflatoxin B_1, B_2, G_1, G_2 and total aflatoxins and OTA, the applicable legislation is Commission Regulation 1881/2006/EC (as amended) (1).
- For ZON, DON, FB_1 and FB_2, and total fumonisins, the applicable legislation is Commission Regulation 1881/2006/EC (as amended) (14).
- For T-2 and HT-2 toxins and the sum of T-2 and HT-2 toxins, the samples have been assessed against the Commission Recommendation 2013/165/EU on the presence of T-2 and HT-2 toxin in cereals and cereal products (15).
- For NIV and FB_3, there are no legislative limits or recommendations. Therefore, the results are for information only.

MAIZE

Aflatoxins

Out of a total of six maize samples analysed for aflatoxins, no sample was found to exceed the levels for AFB_1 and only two samples were found to contain quantifiable (more than 0.2 µg/kg) levels of AFB_1. For AFB_2, AFG_1 and AFG_2 toxins, no sample showed quantifiable levels. All samples were below the 2 µg/kg maximum level set out in Regulation (EC) 1881/2006 as can be seen from Figure 2 below.

Figure 2

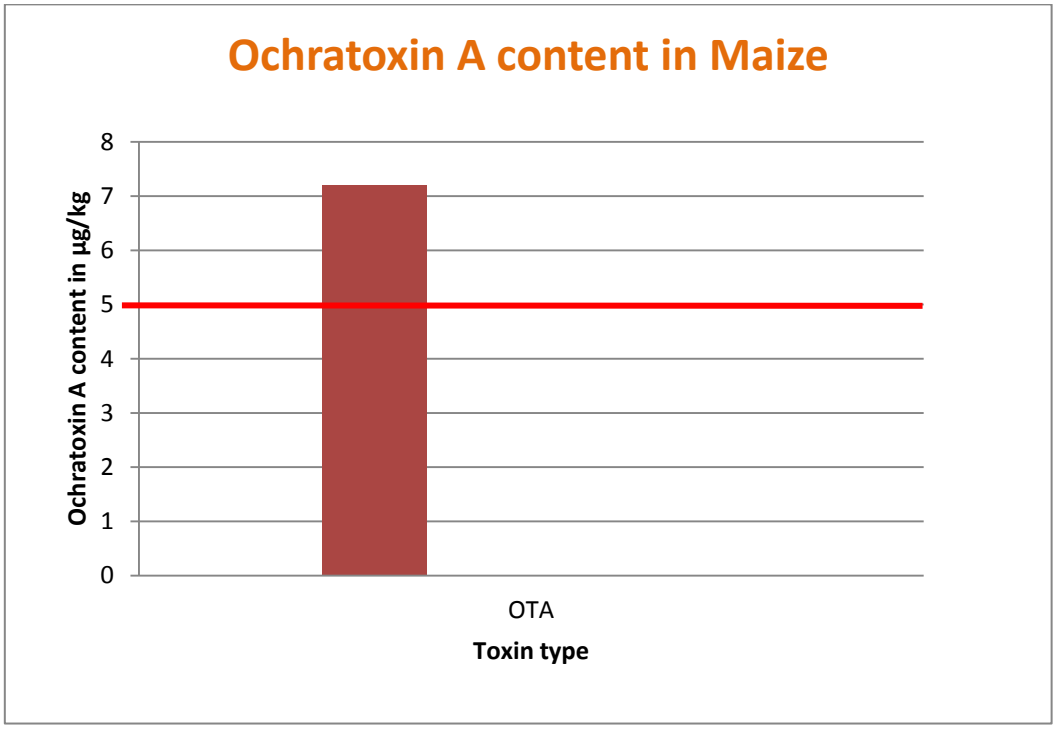


Ochratoxin A

Only one out of the six samples analysed showed an OTA content above the maximum level (5 µg/kg) set out in Regulation (EC) 1881/2006 for unprocessed maize. The remaining samples did not show quantifiable levels (more than 1 µg/kg) of OTA (see Figure 3).

After follow-up with the supplier in relation to the non-compliant sample, it was discovered that the sample had not entered the food chain but the feed chain instead. Therefore, the applicable limit for that sample is 250 µg/kg as set out in Commission Recommendation 2006/576/EC (16) and the sample was compliant.

Figure 3

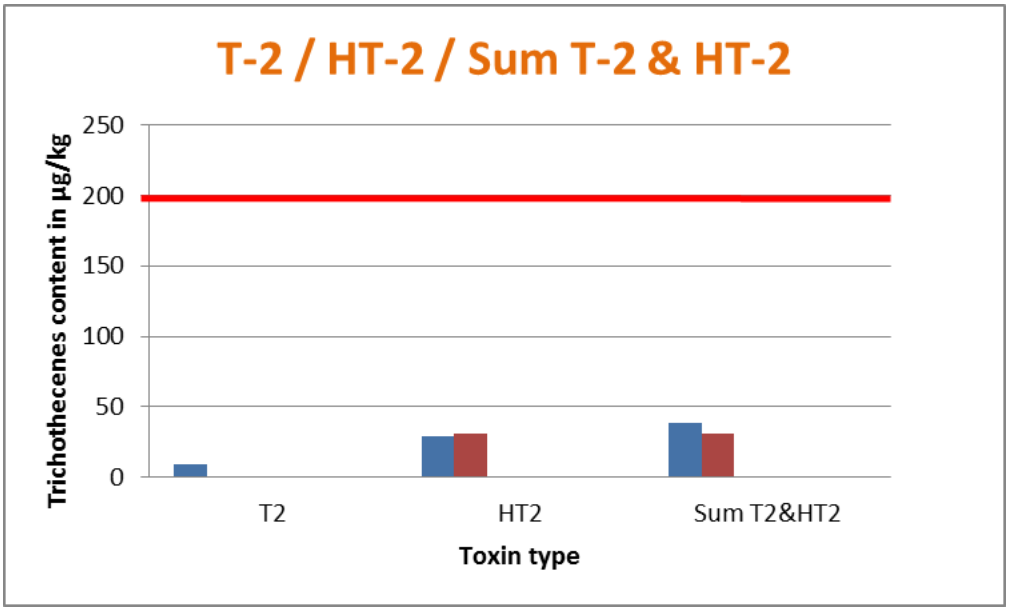


Trichothecenes

There were no regulatory maximum levels for T-2 and HT-2 toxins and their sum, at the time of sampling for this survey. However, more recently, Commission Recommendation 2013/165/EU has set a maximum level of 200 µg/kg for the sum of T-2 and HT-2 toxins. This was used to assess the compliance of the samples. Nevertheless, it should be noted that the results are only indicative as the limits were not applicable at the time of the analysis.

Only two of the samples analysed contained quantifiable levels of toxins (38.6 µg/kg and 30.8 µg/kg respectively) and in all the cases, they were well below the regulatory limits (Figure 4).

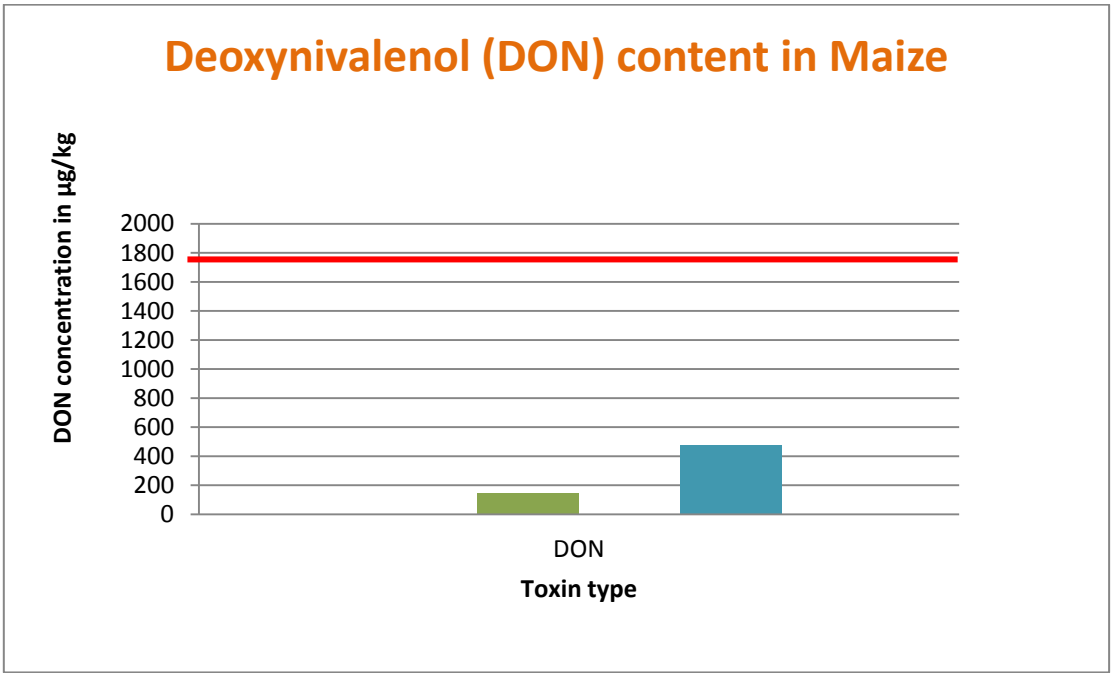
Figure 4



Deoxynivalenol

None of the maize samples analysed showed DON content above the legal limit and only two of them had quantifiable levels of the toxin (145 µg/kg and 475.6 µg/kg). In both cases, the levels found were well below the limit of 1,750 µg/kg (see Figure 5).

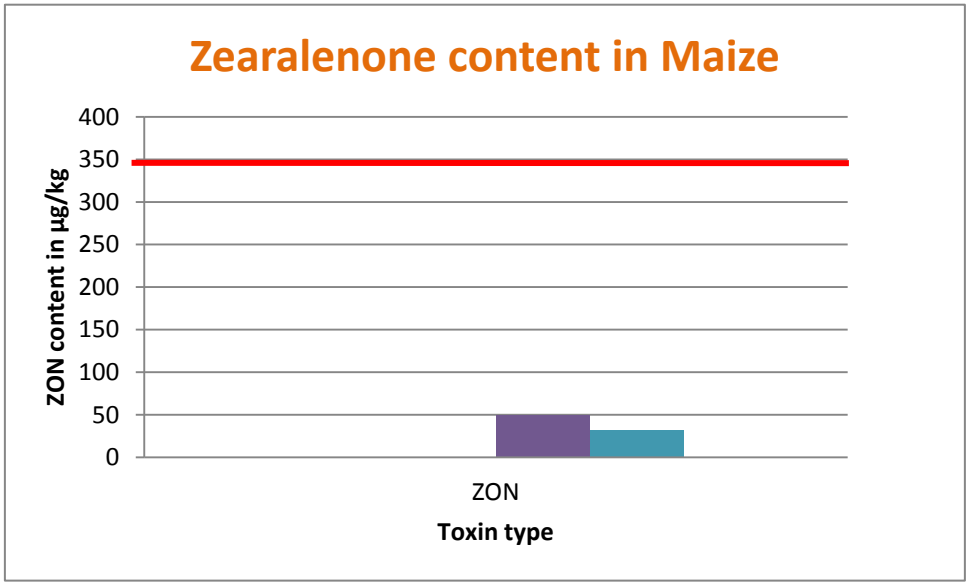
Figure 5



Zearalenone

As for DON, all the samples analysed were well below the legal limit (350 µg/kg) for ZON toxin and only two of the samples actually showed quantifiable levels (32.5 µg/kg and 49.5 µg/kg). See Figure 6.

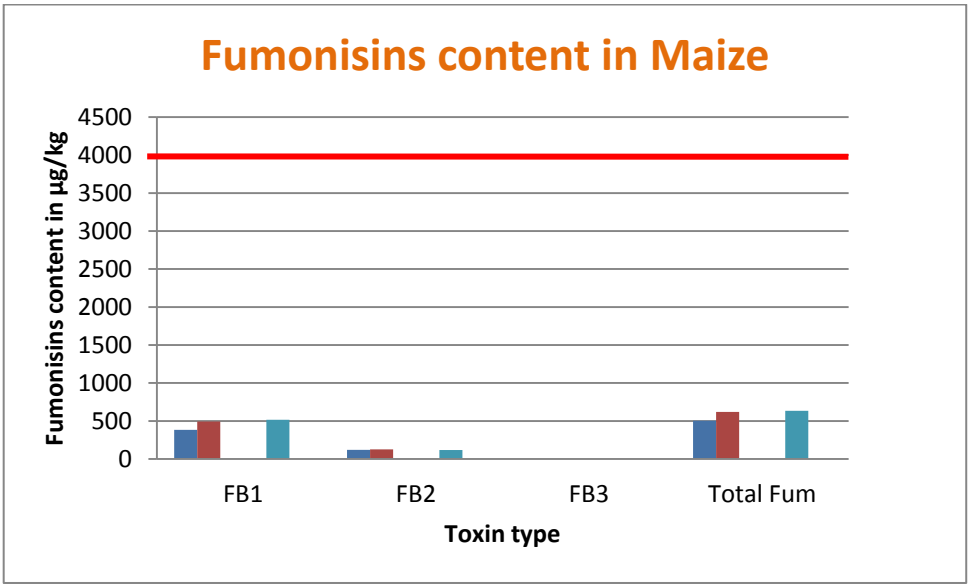
Figure 6



Fumonisins

All samples analysed were well below the regulatory limits (4,000 µg/kg) for total Fumonisins set out in Regulation (EC) 1881/2006 (as amended). These levels were 504.7 µg/kg, 620.4 µg/kg and 635.2 µg/kg.

Figure 7



Nivalenol

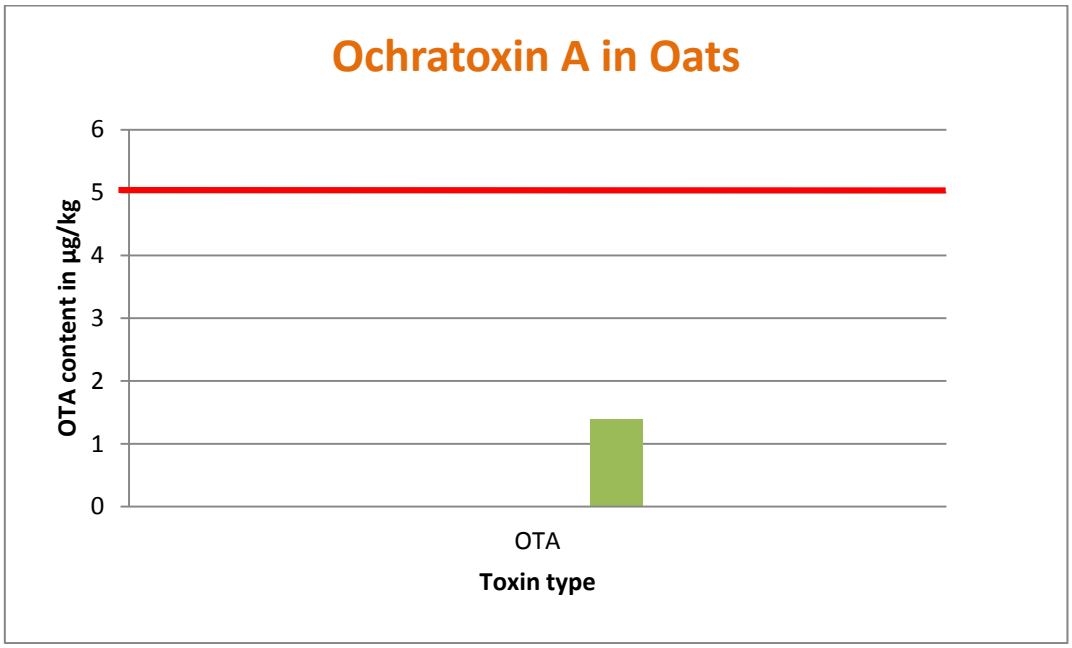
The NIV content was under quantifiable limits for all the samples tested.

OATS

Aflatoxins and Ochratoxin A

No aflatoxins were detected in any of the 14 oat samples and only one sample out of nine showed quantifiable levels (1.4 µg/kg) of OTA that were well below the limit established in Regulation 1881/2006 (5 µg/kg). See Figure 8.

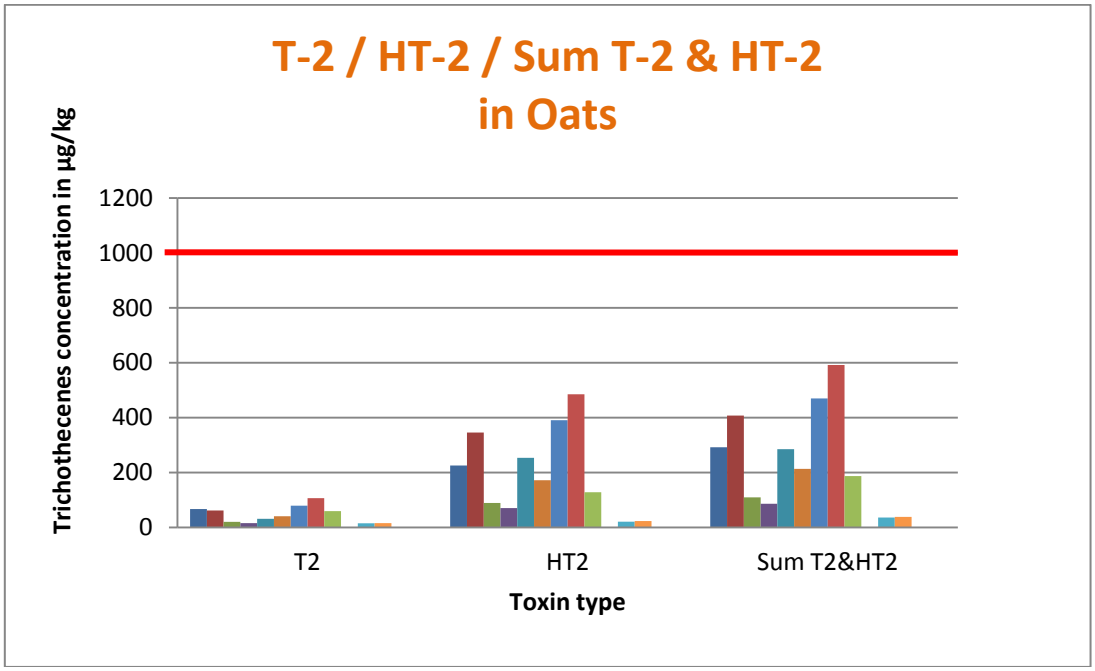
Figure 8



Trichothecenes

Although T-2 and HT-2 toxins where detected in most of the samples (see Figure 9), the sum of T-2 and HT-2 toxins remained low, i.e. under 600 µg/kg, and well below the limit for feed products (2,000 µg/kg). Nevertheless, there was no EC regulatory limit established at the time the samples were collected and the recommended limit set out in Commission Recommendation 2013/165/EU was used for informative purposes in this survey.

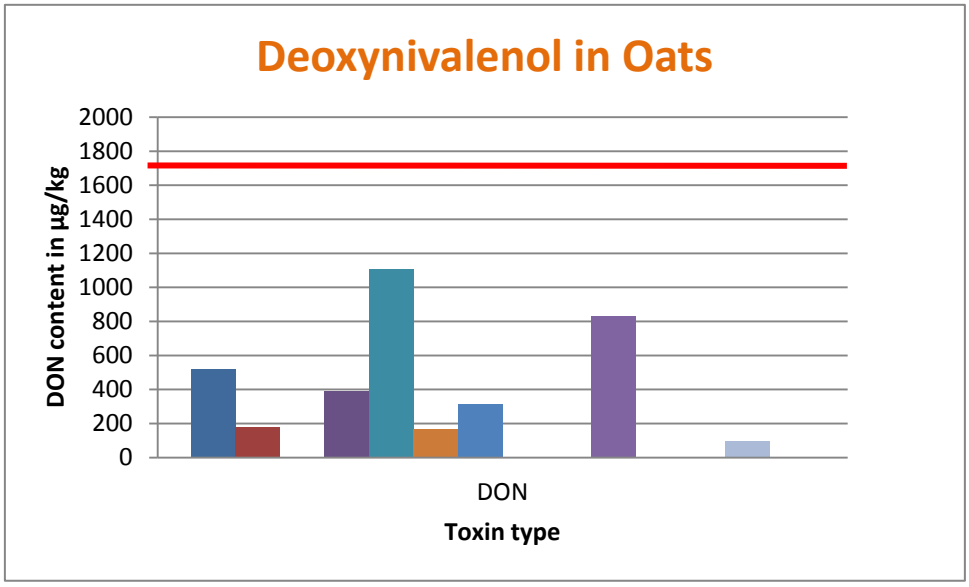
Figure 9



Deoxynivalenol

DON was present in eight of the fourteen samples of oats at levels ranging from 98 µg/kg to 1,105 µg/kg. Regulatory limit for deoxynivalenol in oats is set out in Regulation 1881/2006 (as amended) at 1,750 µg/kg of unprocessed oats with DON levels being below this limit for all of the samples analysed (see Figure 10).

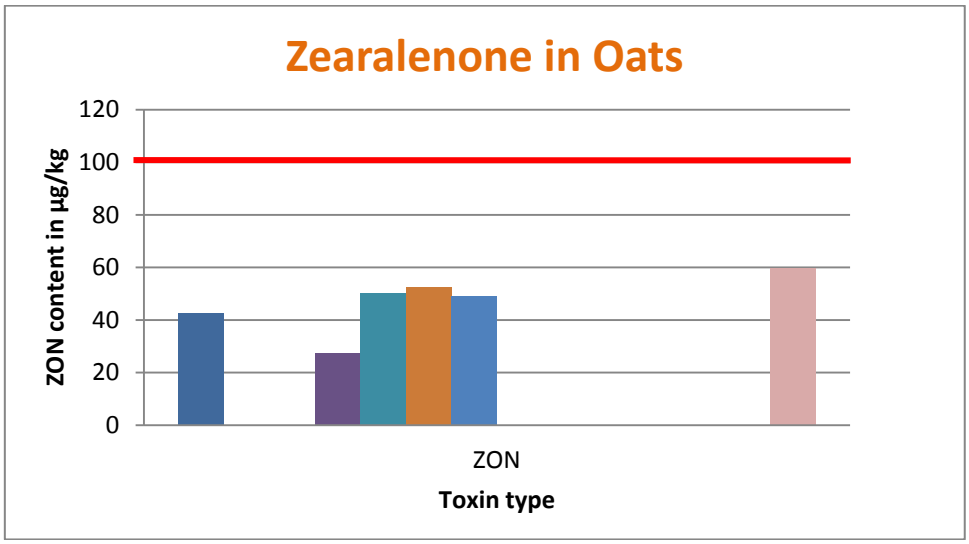
Figure 10



Zearalenone

Only seven out of the fourteen samples of oats analysed contained quantifiable levels of ZON, with the highest level found being 59.7 µg/kg and the lowest 27.2 µg/kg, these being considerably lower than the legal limit of 100 µg/kg of unprocessed oats (see Figure 11).

Figure 11



Fumonisins and Nivalenol

None of the oat samples analysed presented quantifiable amounts of either fumonisin toxins or nivalenol.

BARLEY

Twenty samples of barley were sampled and analysed as part of this mycotoxin survey. The results of which are given below.

Aflatoxins and Ochratoxin A

Regulatory limits for aflatoxins in barley have been established at 2 µg/kg for AFB_1 and 4 µg/kg for the sum of aflatoxins (B_1, B_2, G_1 and G_2) in Regulation (EC) No1881/2006 (as amended) and none of the barley samples analysed contained quantifiable levels of aflatoxins.

Regarding OTA, only one sample contained OTA levels (36.6 µg/kg) that were well above the limit of 5 µg/kg set out in Regulation (EC) No1881/2006 (OTA). Nevertheless, following consultation with the supplier, the sample was deemed compliant as it was intended for use in feed, where the limit for OTA is set out at 250 µg/kg in Commission Recommendation 2006/576/EC.

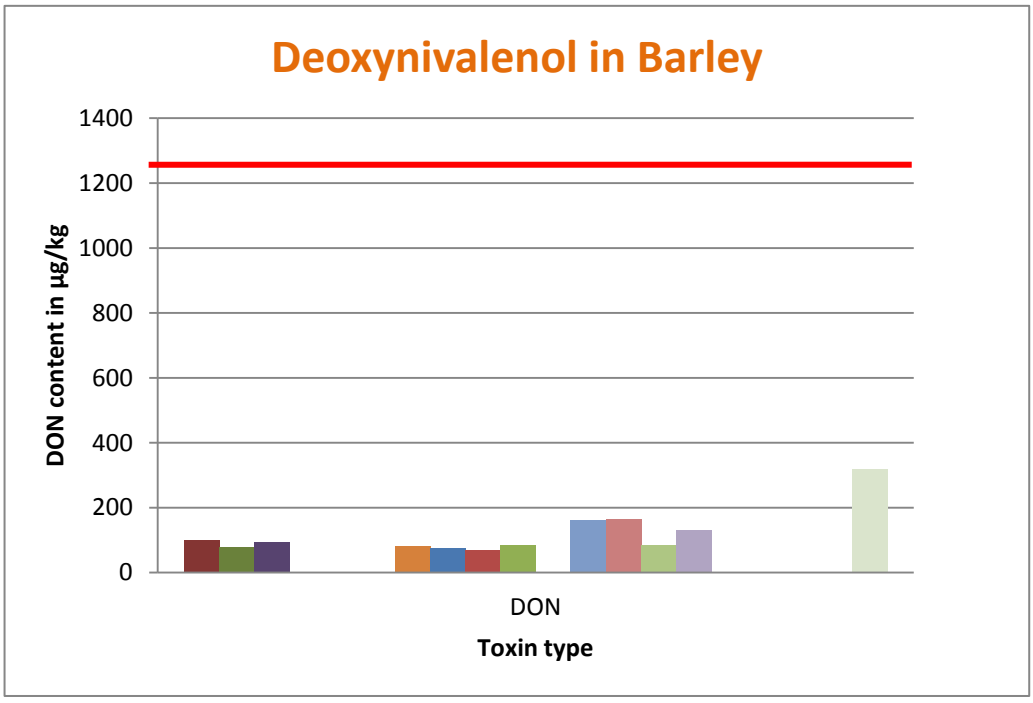
Trichothecenes

As with Aflatoxins and OTA, none of the barley samples analysed showed quantifiable levels of trichothecenes toxins.

Deoxynivalenol

Twelve of the samples of barley contained low levels of DON, which in all cases were below the regulatory limit of 1,250 µg/kg as set out in Regulation 1881/2006 (as amended). See Figure 12.

Figure 12

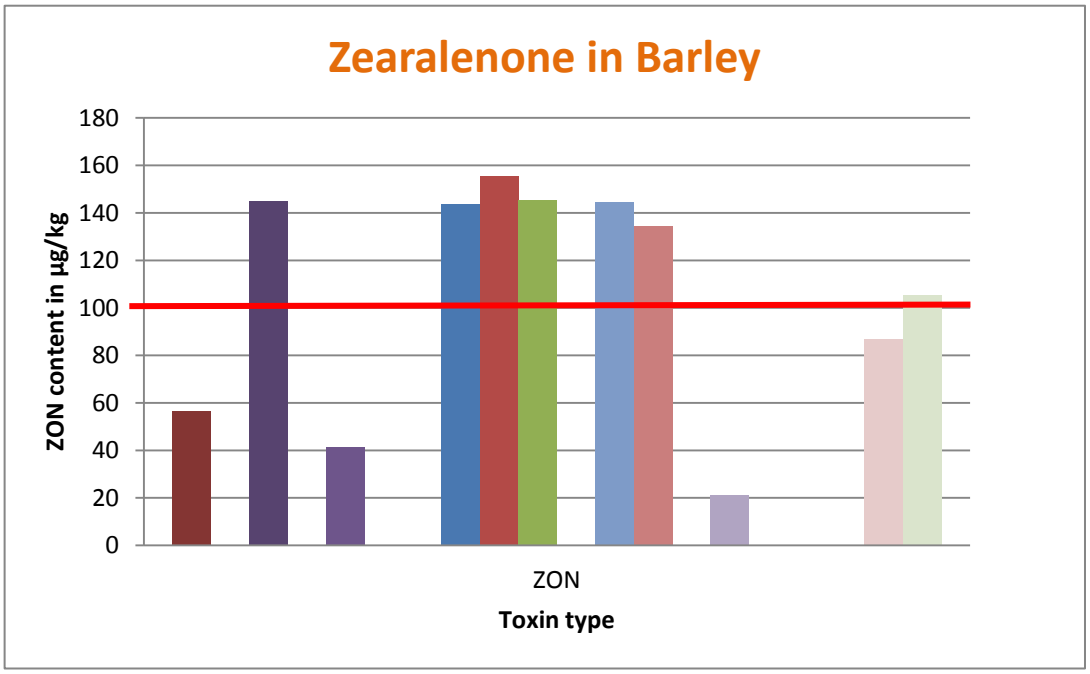


Zearalenone

Six of the twenty samples analysed showed levels above the regulatory limit (100 µg/kg) for ZON in barley as established in Regulation 1126/2007 (see red line in Figure 13). Given that ZON is an oestrogenic mycotoxin with relative low toxicity and that barley is not as widely consumed as wheat or maize, these levels should not pose a threat to human health.

Nevertheless, non-conforming samples were reported for further investigation and following consultation with the supplier, it was found that the samples were destined to the feed chain where the applicable limit was 2,000 µg/kg (Figure 13) as set out in Commission Recommendation 2006/576/EC. Therefore, all samples were compliant and well below the regulatory limits for this purpose.

Figure 13



Fumonisins and Nivalenol

For both fumonisins and NIV toxins, none of the samples contained quantifiable levels.

WHEAT

Aflatoxins and Ochratoxin A

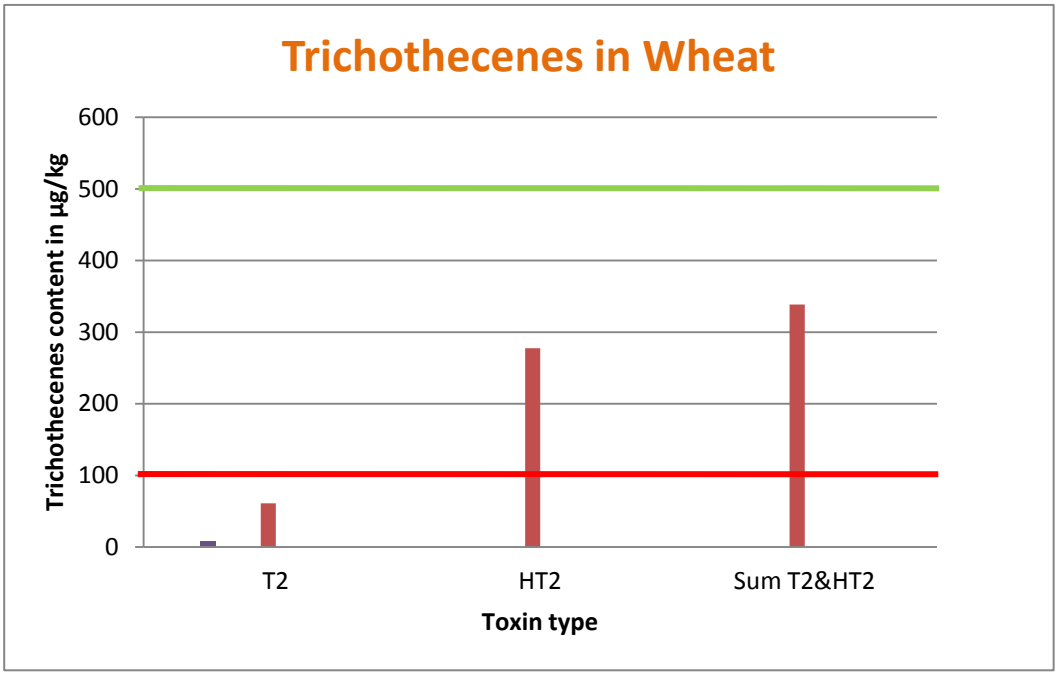
None of the sixteen samples of wheat submitted for analysis contained quantifiable levels of aflatoxins or OTA.

Trichothecenes

One sample out of sixteen was found to be non-compliant for the sum of trichothecenes, with a level of 338.6 µg/kg, which is above the recommended limit of 100 µg/kg set out by Commission Recommendation 2013/165/EU. Nevertheless, as there was no statutory limit in force at the time of sampling and analysis, the following results are for information only. See Figure 14.

Nevertheless, the sample was followed up with the supplier and was found to be destined for use as animal feed, therefore not entering the human food chain directly. According to Commission Recommendation 2013/165/EU of 27 of March 2013, on the presence of T-2 and HT-2 toxin in cereal and cereal products, the limit for the sum of T-2 and HT-2 toxins in cereal product for feed and compound feed is set out at 500 µg/kg (see green line in Figure 14). Bearing this in mind, the sample was subsequently deemed to be compliant for the purposes for which it was to be used.

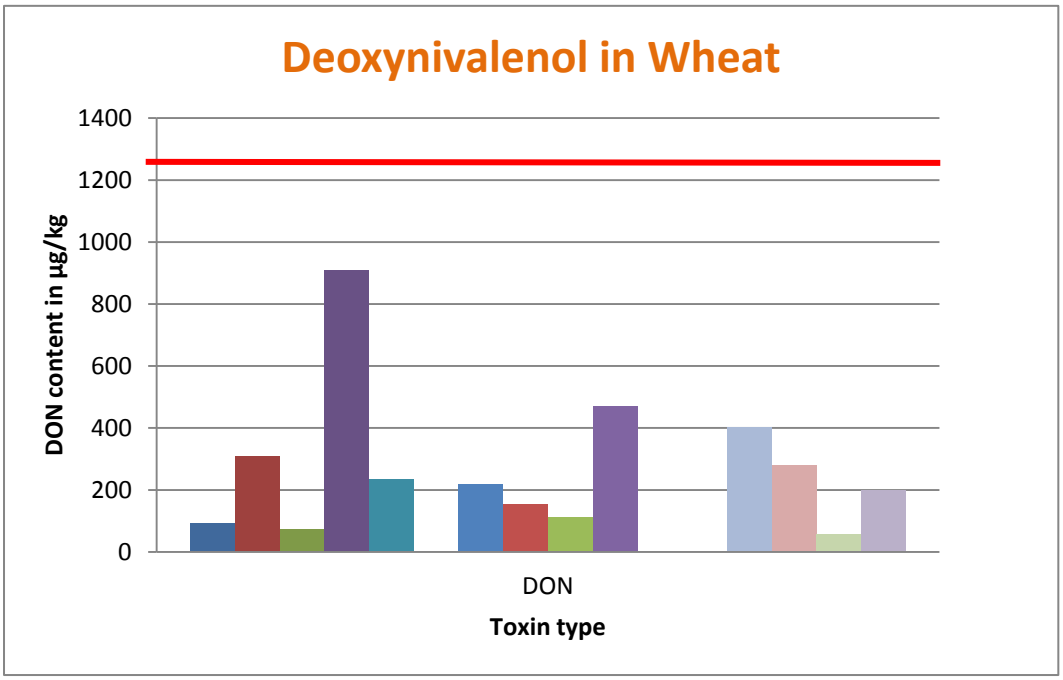
Figure 14



Deoxynivalenol

Thirteen out of the sixteen samples of wheat showed quantifiable contents of DON that ranged between 57.8 µg/kg and 907.1 µg/kg, which are well below the regulatory limit of 1,250 µg/kg set out in Regulation 1881/2006 (as amended) (see Figure 15).

Figure 15

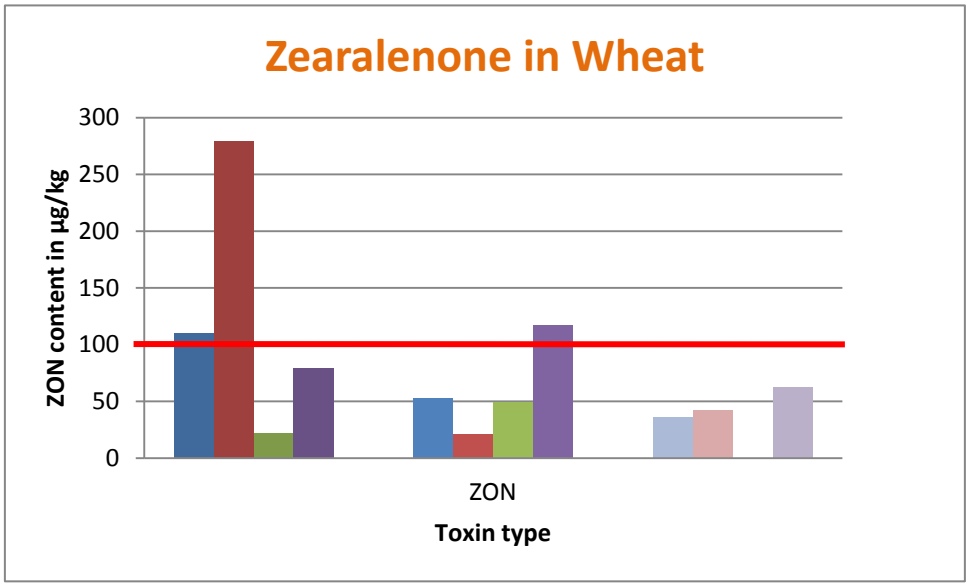


Zearalenone

After the application of the measurement of uncertainty (MU) for the analytical method used, only one sample of wheat showed a level for zearalenone (278.9 µg/kg) that was non-compliant and more than 2.5 times the level set out in Regulation 1881/2006 as amended (100 µg/kg) for this substance (see Figure 16).

As with the barley cases, ZON is considered to have relatively low toxicity. Non-compliant samples were reported and followed up on with the supplier and all of them were also found to be destined for animal feed, where the level of ZON permitted in accordance with Commission Regulation 2006/576/EC is 2,000 µg/kg.

Figure 16



Fusarium toxins and Nivalenol

None of the analysed samples of wheat showed quantifiable levels for the sum of fusarium toxins and nivalenol.

CONCLUSION

Of a total of 56 samples analysed, nine (16%) did not comply with the regulatory limits set out for food. After following up with the different suppliers, it was found that in all cases of non-compliant samples, they had entered the animal feed chain, and these samples were therefore deemed to be compliant under the applicable regulatory limits for feed. It should be noted that the samples in this study were taken as non-statutory surveillance samples. Therefore, the sample size was smaller than that required under Commission Regulation (EC) 401/2006.

Most samples (84%) showed quantifiable levels for one or more of the analysed mycotoxins (although all levels remained below the legislative limits), with the prevalence for each particular mycotoxin being as follows:

- Two (4%) of the 56 samples showed quantifiable levels for aflatoxins, 4 (7%) for ochratoxin A and 3 (5%) for fumonisins
- The number of samples that were found to have quantifiable levels of mycotoxins increased for the mycotoxins trichothecenes, zearalenone and deoxynivalenol, with 14 (25%), 30 (54%) and 35 (63%) samples respectively testing positive
- No sample was found to contain levels of Nivalenol and in total, 10 (16%) samples had no quantifiable levels of any of the mycotoxins analysed

This survey does not raise any safety concerns and consequently, consumers do not need to change their diets as a result of these findings.

The production of mycotoxins-free cereals is still very difficult and far from being achieved but the FSAI is of the opinion that provided the levels of mycotoxins are kept to the lowest levels achievable, the benefits of eating cereal-based food outweigh any possible risk to health.

Despite the encouraging results, the FSAI will continue to work with the food industry to help reduce mycotoxins in foodstuff and protect the consumers ensuring that where they are present, levels of mycotoxins are within the statutory limits.

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ACKNOWLEDGEMENTS

Special thanks to the Irish Grain and Feed Association and Dublin Public Analyst Laboratory for facilitating the sampling and analysis of the cereals considered in this survey.

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