Deoxynivalenol (DON) is a mycotoxin produced by fungi of the Fusarium genus, i.e. *Fusarium culmorum* and *Fusarium graminearum*, which are abundant in various cereal crops (wheat, maize, barley, oats, and rye) and processed grains (malt, beer and bread). Chemically it belongs to trichothecenes. In contaminated cereals 3- and 15-acetyl DON can occur in significant amounts (10 – 20%) concomitantly with DON. The fungi producing trichothecenes are soil fungi, and are important plant pathogens which grow on the crop in the field.¹

Deoxynivalenol – a Mycotoxin of Increasing Concern

by Dr. Elisabeth Pichler

Fusarium pathogens are known to infect plants primarily during the flowering period. Severity of fungal infection can be related to climatic conditions such as warm springs and high rainfalls during the early flowering period. Besides climatic conditions, some agricultural practices influence the infection rate and the subsequent mycotoxin production: For wheat, a major source of infection is plant parts of maize that remained on the field from the previous season. Cultivation that reverses the soil and removes infectious plant parts helps reduce infection pressure. For maize, the situation is not that clear. It appears that insect damage is a major influence here. The main toxin production by *Fusarium species* happens in the field, but studies have shown that DON contamination might also increase due to unfavorable storage conditions: *Fusarium species* need 17-19% moisture content for growth, numbers that are frequently reached in Europe during harvest. Thus, harvest and storage conditions play an important role in DON contamination and must be taken into consideration if strategies to reduce DON contamination are discussed.²
Numerous studies conducted within the last 25 years have shown that the immunological effect of DON is paradox and highly complex: On the one hand many studies have clearly shown that high dosages of DON lead to immune suppression in different animal species. On the other hand, it has been shown that low doses of DON increase the resistance to certain pathogens and elevate serum IgA levels. The stimulatory effects of low doses of trichothecenes apparently relate to their ability to induce immune- and inflammation-associated genes. In contrast, it appears that the suppressive effects of trichothecenes on leucocyte function are intimately linked with the induction of apoptosis as has been demonstrated in macrophages, T cells and B cells both in vivo and in vitro. Based on data on Deoxynivalenol, Nivalenol, T-2 and HT-2 from recent opinions prepared by the European Commission Scientific Committee on Food (SCF) and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) levels for Tolerable Daily Intakes (TDI) of 1, 0.7 and 0.06 g/kg b.w. were established for DON, NIV and the sum of T-2 and HT-2, respectively. The TDI’s for NIV, T-2 and HT-2 were made temporary because of deficiencies in the database.

Deoxynivalenol is the mycotoxin that is most prevalent in European crops. According to the final report of SCOOP task 3.2.10, comprising information on the occurrence of Fusarium toxins in food and assessment of the dietary intake by the population of EU member states, a considerable number of data was provided, with most of them originating from North European countries.

- Among the individual commodities, wheat has been investigated more widely in comparison with other grains.
- Weighed mean 1 ranged from 37 µg/kg for barley to 594 µg/kg for corn.
- Weighed mean 2 (all positive samples) ranged from 95 µg/kg for rye to 660 µg/kg for corn.
- Corn showed the highest level of contamination, with 89% of positive samples.

Many countries have established regulatory limits for DON and recently the European Union released new regulations as well.

### Regulatory updates – new regulation in the EU.

Regulatory levels for DON, according to COMMISSION REGULATION (EC) No. 856/2005:

<table>
<thead>
<tr>
<th>Product*</th>
<th>Maximum level [µg/kg]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unprocessed cereals other than durum wheat, oats and maize</td>
<td>1250</td>
</tr>
<tr>
<td>Unprocessed durum wheat and oats</td>
<td>1750</td>
</tr>
<tr>
<td>Unprocessed maize</td>
<td>300</td>
</tr>
<tr>
<td>Cereal flour, including maize flour, maize grits and maize meal</td>
<td>750</td>
</tr>
<tr>
<td>Bread, pastries, biscuits, cereal snacks and breakfast cereals</td>
<td>500</td>
</tr>
<tr>
<td>Pasta (dry)</td>
<td>750</td>
</tr>
<tr>
<td>Processed cereal-based food for infants and young children and baby food</td>
<td>200</td>
</tr>
</tbody>
</table>

* more details and specification can be looked up in Commission regulation No 856/2005
** if no specific level is fixed before 1 July 2007, the level of 1750µg/kg will apply thereafter to maize referred to in this point.

**Impact of DON and the new regulation on the bio-ethanol producing industry and on breweries.**

Bio-ethanol producers have to check for mycotoxin contamination including DON levels. This is not only due to new regulatory limits, but because of by-products being distributed as feed. During the alcohol-fermentation process, concentration of mycotoxins occurs, leading to increased levels of the toxin. To obtain high quality feedstuff, it is critical that the used grain like wheat enters the process with a very low mycotoxin contamination. Südzucker Bioethanol GmbH, a subsidiary of Südzucker AG, one of the largest sugar refining companies in the EU, has set the following specification regarding mycotoxins for the wheat they buy from contract farmers:

Low mycotoxin levels of lower than:
- Deoxynivalenol < 500 µg/kg
- Zearalenone < 50 µg/kg
- Ochratoxin A < 50 µg/kg

Trichothecenes might also have an impact on the fermentation process of beer directly. A Brazilian study showed that contamination of malt with Deoxynivalenol and/or T-2 at different ratios (up to 500 µg/kg) influenced the profile of the alcohols produced by Saccharomyces cerevisiae during the fermentation process. Especially the profile of methyl and isoamyl alcohols.

Other authors described that “in addition, Fusarium infestation in barley and malt may be associated with “gushing” in the resultant beer,” Gushing is defined as the quick uncontrolled and spontaneous over-foaming immediately after opening a bottle or can. Fusarium graminearum, Fusarium culmorum and Fusarium poe have all been named as active gushing inducers. The production of mycotoxins may possibly occur parallel to the production of the components responsible for gushing. Production of mycotoxins during malting was studied for DON. It was revealed that the final concentration of DON in malt was approximately the same or lower than the concentration detected in barley.

**Impact of new DON regulation in milling industry**

Of all industries, the milling industry is probably affected the most by the new regulation. Products have to comply with the regulatory levels, which will also put pressure on grain suppliers. As an example, a letter from the National Associations of the Milling (Italmopa) and Cereals (Anacer) Industries to the Office of Agricultural Affairs in Rom shall be mentioned: It expresses concern that the time period shortly after the first of July (date of implementation of the new EU regulation) might bear problems. In this time period, flour produced from wheat imported before the new regulation was put in force would be used to produce bread and other products subject to the new regulation, which could exceed the new regulatory limits.
They also refer to the issue that shipments loaded and tested in the USA will have to be furnished with certification attesting the observance of the EC limits based on tests performed in conformity with the requirements laid down in EC directive 2005/38.

**Impact of DON in swine and poultry industry**

Although the new regulation on *Fusarium* toxins mainly affects the food sector and the levels are rather high considering the amount of grain in the whole feed ratio, they will also have an impact on animal producers. Contamination of feedstuffs with mycotoxins occur despite the most strenuous efforts on prevention. The economic consequences are felt by crop- and animal producers as well as by food- and feed processors.

In cases where mycotoxin manifestation was evident, the first and most practical approach has been blending of low contaminated grain with grain above the limits, thus lowering the average contamination levels to the accepted standards. But this approach is prohibited in the European Union. Since all mycotoxins are quite stable substances, no physical or chemical treatment can be applied under practical field conditions, without altering the nutritive value of the grain or causing high cost implications. Therefore the danger is evident that “low quality” grain could be used for preparation of feed, especially in so-called home-mixing countries where the animal producers are preparing their feedstuffs on site as an adequate control will not be possible.

Although scientific literature offers a broad variety of information on the effects of individual mycotoxins in various animal species, it is the multiple mycotoxin contamination that matters in the pig and poultry industry most, as it refers to the naturally occurring circumstances. Poor livestock performance and disease symptoms observed in commercial operations may be due to the synergistic interactions between multiple mycotoxins. Multi-toxin occurrence is one important explanation for divergences in effect-levels described in scientific literature, where defined, mostly purified mycotoxins are used as source of contamination, in comparison to effective levels observed in practice, where apparently lower mycotoxin contaminations often cause more severe effects.

*This chapter was generously contributed by Ursula Hofstetter, Biomin GmbH.*

**Sampling prior to DON-testing.**

It is generally recognized that sampling is the most crucial part in mycotoxin testing and that without a good sampling plan results are worthless. Sampling problems with Deoxynivalenol are less prevalent than with aflatoxins, but still a factor to be taken into consideration. Favorable factors for DON sampling is that the effective concentrations and the regulatory limits are ten to hundred times higher than those for aflatoxins. In addition to the new regulatory limits for *Fusarium* toxins, the European Union has also published a revised directive regarding sampling. It covers sampling plans for many different matrices like cereals, cereal product, spices, milk, baby food etc. It gives recommendations depending on the size of the total lot as well as depending on the physical conditions.

**Ways of testing for DON: optimized solution for each industry.**

- **FluoroQuant® DON Plus – Single sample testing**
  
  This quantitative test is optimally used in situations where single samples have to be tested within a short period of time. The testing of incoming truck loads during harvest season is such a situation. Romer Labs® is now launching the new FluoroQuant® DON Plus test kit that allows quantification within approx. 10 minutes. The limit of quantification is as low as 0.2 ppm and it is validated for wheat, barley, malted barley and corn. Since the test is not antibody based, it is very robust and can be used by untrained personnel under rough conditions.

- **AgraQuant ELISA - Multi sample testing**
  
  AgraQuant® ELISAs are optimally used, when many samples can be tested at once. By using the AgraQuant® DON test kit, quantitative results can be obtained within 20 minutes. With a limit of detection of 0.2 ppm and a quantification range of 0.25 to 5 ppm, the test is ideally designed for checking commodities for their compliance with EU regulatory limits. Up to 96 samples and standards can be quantified at once. This immunological test can be used for many commodities such as corn, wheat, barley, etc.

- **TLC/HPLC – MycoSep® – Biopure® Mycotoxin Standards**
  
  Chromatographic methods are usually reference methods used to confirm positive screening results. Romer Labs® offers several TLC, HPLC, and GC methods for DON and other trichotheccenes, such as nivalenol, fusarenon X, 3- and 15-acetyldeoxynivalenol, or the type A trichotheccenes T-2 toxin, HT-2 toxin, DAS etc. Generally, clean up for all mentioned chromatographic methods can be done by MycoSep® columns (MycoSep® 227 Trich+, or MycoSep® 225 Trich).
After a concentration step, the sample is subjected to chromatographic separation and quantification by use of UV-detection or mass spectrometry. Numerous publications describe clean up and subsequent determination. 

Biopure® mycotoxin reference standards help to increase quality of the analytical procedure substantially. Biopure® mycotoxin calibrants are traceable to SI system and come with full documentation of the certified concentration value and the uncertainty statement according to ISO Guides 31 & 35. 

Biopure® also now offers stable isotope labeled internal standards for reliable quantification of mycotoxins with mass spectrometry. In the case of the most important trichothecenes Deoxynivalenol and T-2 Toxin, the most suitable internal standards are U-[13C15]-Deoxynivalenol and U-[13C24]-T-2 Toxin, which automatically correct for errors and losses during sample preparation steps and subsequent ionization process in the mass spectrometer.

Conclusions

After many years of discussion, Fusarium toxins are now subjected to EU regulation. These new regulatory limits do not only impact industries within Europe, but also suppliers outside Europe. Thus, we are dealing with a topic of worldwide importance. Only the right choice of screening and reference methods will help the involved industries to protect their interests at reasonable costs.

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