

Maximum Group Levels for DON, 3-acetyl-DON, 15-acetyl-DON and DON-3-glucoside

Occurrence, toxicity, analysis, maximum levels

A. What is Deoxynivalenol?

Deoxynivalenol (DON) belongs to the group of so-called mycotoxins. They are built as metabolic products of molds on foods such as cereals, oily seeds, fruits and cocoa. Because of their partially extreme toxicity and carcinogenicity, mycotoxins are also considered a potential health risk in trace levels and are therefore undesirable on foods. About 400 mycotoxins from about 350 types of molds were identified to date. The most important toxin building molds are the genera *Aspergillus*, *Penicillium*, *Fusarium*, *Alternarium* and *Claviceps*. Besides T2 toxin and HT2 toxin, DON is one of the *Fusarium* toxins and has a so-called trichothecene structure. This large group of mycotoxins is characterised by the common 12,13-spiroepoxy-sesquiterpenoid basic structure. DON can mainly be detected on domestic cereals such as wheat and corn. There were also DON findings on cereal products such as bread, pasta and beer as well as in oilseeds. DON is one of the most frequently studied mycotoxins worldwide and is often found together with other mycotoxins, especially with other fusarium toxins.

B. What other forms of DON are relevant and why?

In Europe, the structurally related metabolites of DON, 3-acetyl-deoxynivalenol (3-Ac-DON) and 15-acetyl-deoxynivalenol (15-Ac-DON) often occur. They are produced by plant pathogenic fungi of the genus *Fusarium*, which grow on cereals in the field, preferably in temperate climates. In addition to the acetylated metabolites, DON-3-glucoside is a modified form of DON (also called masked DON) and considered the

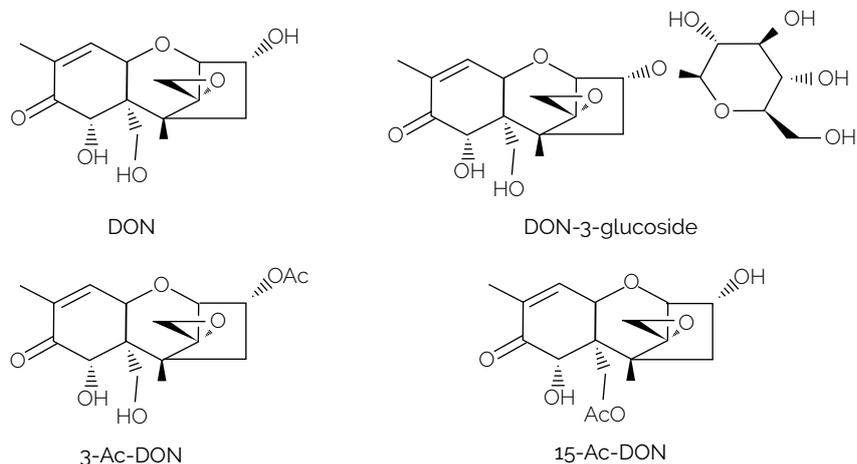


Figure 1. Structures of DON, 3-Ac-DON, 15-Ac-DON and DON-3-glucoside

most important plant metabolite of DON. These four forms, DON, 3-Ac-DON, 15-Ac-DON and DON-3-glucoside are predominantly found on cereal grains such as wheat, barley, oats, rye and corn. Their structures are shown in figure 1.

C. Are DON and its modified forms toxicologically relevant?

The epoxy function seems to be responsible for the toxic effects of DON. Health damage can be observed in both humans and animals. Type B trichothecenes such as DON are considered to be effective inhibitors of protein biosynthesis through their epoxide functionality binding to the DNA. In addition, the chronic intake of DON leads to an increased susceptibility to infectious diseases because it has immunosuppressive properties. Taking into account all available toxicological and toxicokinetic information, the CONTAM Panel 2017 found that 3-Ac-DON and 15-Ac-DON were largely deacetylated to DON prior to systemic distribution in the body, so that they trigger the same acute and chronic effects compared to DON. The available data also indicate that DON-3-glucoside is degraded into DON by bacteria in the gastrointestinal tract

and by that is also distributed and metabolized in a similar way to DON.

D. How are DON and its modified forms analyzed?

The analytical methods for DON, 3-Ac-DON, 15-Ac-DON and DON-3-glucoside are already well established and usable for grain, food, animal feed and biological samples. The exact quantification of DON, its acetylated forms and DON-3-glucoside is mostly done by liquid chromatography in combination with (tandem) mass spectrometry, currently often by means of a multi-analytical approach. Proficiency and round robin tests have shown that there is considerable analytical variability in the determination of DON-3-glucoside. Direct approaches (which require their own standards) and indirect approaches (based on the degradation to DON) have been reported for the determination of DON-3-glucoside. Immunochemical methods for DON offer quick and economical alternatives to chromatography, but cross-reactivity and matrix effects have not yet been fully considered. Recent advances in biomarker research have enabled the determination of DON and its metabolites in urine,

Table 1. Maximum levels for DON and the total DON + mod. forms in food under discussion

Food category	Current maximum residue level (µg/kg)	Level based on P95 (µg/kg)	Level for the sum DON + mod. forms (µg/kg)
Unprocessed cereals	1250	1000	1500
Unprocessed durum wheat and oats	1750	1250	1750
Unprocessed corn	1750	1250	1750
Cereals intended for direct human consumption	750	Barley, rye, millet: 750 Other cereals and ground products: 500	Barley, rye, millet: 1000 Other cereals and ground products: 750
Pasta (dry)	750	400	600
Bread, pastries, cookies, cereal snacks, breakfast cereals	500	400	600
Processed cereal-based foods and baby foods for infants and young children	200	100	150

especially as DON glucuronides. However, the commercial sources for the standards of DON glucuronides are scarce and so far no (certified) reference materials are available.

E. EFSA data collection

EFSA published an updated DON risk assessment in 2017. The so-called "modified forms" (formerly also: "masked mycotoxins") should be included and it should be checked whether toxicological reference values – such as TDI (tolerable daily intake; measure for the chronic risk) and ARfD (acute reference dose; measure for the acute risk) – can be derived for the group of mycotoxin plus its modified forms.

The risk assessment also included an exposure assessment. EFSA's data collection contained a total of more than 21,000 data for Deoxynivalenol and its metabolites from 2007 to 2014. Depending on the analyte, this data included up to 96% results that were below LOD or LOQ. Samples with the highest concentrations of DON and the sum of DON, the acetylated forms and DON-3-glucoside were mainly food for special medical purposes and cereals and cereal products. Since DON, 3-Ac-DON, 15-Ac-DON and DON-3-glucoside mostly occur in the outer layers of the grain, process steps such as cleaning, sorting, sieving and peeling cereals lead to significant increases in the concentration of these toxins in grain by-products, e.g. bran.

The mean acute human exposure for the sum of DON, 3-Ac-DON, 15-Ac-DON and DON-3-glucoside ranged from 0.2 to 2.9 µg/kg body weight in 39 different nutritional studies over all

age groups. The 95th percentile ranged from 0.7 to 6.7 µg/kg body weight per day. The mean chronic human exposure to the sum of DON, 3-Ac-DON, 15-Ac-DON and DON-3-glucoside resulted between 0.2 and 2.0 µg/kg body weight per day in 33 different nutritional surveys over all age groups, the 95th percentile ranged from 0.5 to 3.7 µg/kg body weight per day. In both cases, infants had the highest mean nutritional exposure.

The highest contributions to the exposure of the sum of DON, 3-Ac-DON, 15-Ac-DON and DON-3-glucoside come from cereal-based products, in particular "bread and rolls", "fine baked goods" and "pasta (raw)". Based on these data, EFSA set the TDI of 1 µg/kg body weight and day for DON as a group TDI for the sum of DON, 3-acetyl-DON, 15-acetyl-DON and DON-3-glucoside. A group ARfD of 8 µg/kg body weight was also introduced covering the acute risk. For the group consisting of DON, 3-acetyl-DON, 15-acetyl-DON and DON-3-glucoside, no acute risk is to be expected from levels as found in foodstuffs, however exceedances of the group TDI are possible for frequent consumers and children below 10 years.

F. What is the current legal situation regarding DON and its modified forms?

For Deoxynivalenol, currently different maximum levels exist in the EU depending on the food matrix, which are defined in the European Contaminants Regulation VO (EG) No. 1881/2006. They apply, among other things, to cereals and cereal products, such as bread and pasta.

For various food categories, including cereals and cereal products such as pasta, bread, breakfast cereals and cereal-based products for infants and young children, maximum levels for DON and the sum of DON, 3-acetyl-DON, 15-acetyl-DON and DON-3-glucoside are currently discussed at EU level based on the updated EFSA risk assessment and summarized in table 1.

The current working document regarding DON of the »Working Group Agricultural Contaminants« of the EU Commission and the member states cited here is a first proposal. Herein, the maximum level for DON is to be reduced to the 95th percentile based on the EFSA data collection, but extended to the sum levels for 3-acetyl-DON, 15-acetyl-DON and DON-3-glucoside, which might represent up to 35% of the total amount according to the EU Commission.

Currently, an analysis method for the determination of DON and its metabolites is developed at the LCI.



Food Chemistry Institute of the Association of the German Confectionery Industry

Institute Director:
Dr. Frank Heckel
Adamsstraße 52-54
51063 Köln

phone: +49 221 62 30 61
fax: +49 221 61 04 77
web: www.lci-koeln.de