codex alimentarius commission

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

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DISCUSSION PAPER ON DEOXYNIVALENOL

Background

1. The 33rd session of the Codex Committee on Food Additives and Contaminants agreed that a drafting group under the direction of Belgium and with assistance of Canada, Denmark, Germany, the Netherlands, Switzerland, the United States and the EC, would elaborate a Discussion Paper on Deoxynivalenol for consideration at its next Session (ALINORM 01/12A para. 197).

2. Deoxynivalenol or DON or vomitoxin belongs to the group of trichothecenes, mycotoxins produced by *Fusarium* species, especially *F. graminearum* and *F. culmorum* which are pathogens of several cereal grains. Thrichothecenes are sesquiterpenoid compounds.

3. DON is a well-known contaminant of cereals and cereal products.

Screening and analytical methods

4. The association between high DON concentration and the presence of kernels showing the characteristic scab-like appearance generally associated with *Fusarium*-damage is well known within the wheat industry. This raises the possibility of using visual grading parameters of percentage of *Fusarium*-damaged kernels (percentage of scab), or perhaps percentage of total damaged kernels to manage the safety of commercial shipments. A common misconception has existed in the grain trade that percentage *Fusarium*-damaged kernels can be used as a reliable basis for calculating DON concentration once the ratio of DON concentration to percent *Fusarium*-damaged kernels is known. Canadian studies showed that the potential for error is very large and that the relationships between DON concentration and percent *Fusarium*-damaged kernels are neither strong enough nor robust enough to predict DON in individual samples with a high degree of accuracy and precision. The potential success of a visually based management system for dealing with *Fusarium* head blight in wheat requires that grain inspectors consistently recognise symptoms in infected kernels, make reliable assessments of *Fusarium* damage, and accurately quantify the level of infection.

5. Sampling of bulk commodities is a major factor for ensuring that test results accurately reflect the mean concentration of DON in the bulk. However, when effective sampling techniques are employed, errors associated with sample preparation and the analytical method may be greater than errors due to sampling. In one study cited by JECFA, for a batch concentration of deoxynivalenol of 5.0 mg/kg in wheat (sample size 0.454 kg), the coefficient of variation was 6.3% for sampling, 10% for sample preparation and 6.3% for analytical steps. Total coefficient of variation was 13% (ref 2).

6. Near-infrared spectroscopy holds promise for rapid DON testing (ref 3). Correlations ranging from 0.70 to 0.93 between DON levels predicted by near-infrared spectroscopy on both ground samples and whole samples, to DON levels determined by GC technique for various hard and soft wheats, were reported. DON

concentrations predicted by near-infrared testing of ground samples were in closer agreement with levels determined by GC-MS than for near-infrared testing of whole samples.

7. Analytical methods that are available for DON include GC-ECD, GC-MS, LC-UV, LC-fluorescence, LC-MS, TLC, ELISA and immunoaffinity column-fluorescence. Critical evaluations of chromatographic methods currently available for the analysis of DON in cereals were recently published (refs 4, 5). The results from a recent international interlaboratory study of an analytical method for DON and zearalenone in agricultural commodities revealed the desirability for further improvements in analytical procedures for these toxins in order to obtain more accurate measurements (ref 6). DON reference standards are often purchased as crystalline material or thin film therefore before use as calibrants, they are prepared gravimetrically in an organic solvent and stored in a freezer. The stability of DON in various organic solvents was recently studied, and it was observed that acetonitrile was the most suitable solvent for long-term storage of DON as a reference standard (ref 7).

8. Wide availability of reference standards and regular international comparative studies are needed to ensure improved internal and external quality assurances. The Institute for Reference Materials and Measurements, Joint Research Centre of the European Commission, makes available BCR reference materials of DON in maize flour and wheat flour (ref 8). The Food Analysis Performance and Assessment Scheme (FAPAS) from the UK includes deoxynivalenol in wheat flour for 2001-2002 for testing laboratory proficiency. FAPAS has test material for DON in wheat which is available for use by laboratories for quality assurance purposes.

9. A method of analysis for the determination of deoxynivalenol and other trichothecenes in cereals is under discussion for standardisation within the European Committee for Standardisation (CEN). It is a GC-ECD method including extraction with acetonitrile/water and clean-up and derivatisation.

Occurrence

10. Surveys have shown that deoxynivalenol occurs frequently in grains such as wheat, barley, and maize and also in oats, rice, rye, sorghum, and triticale. The types of wheat affected by DON include both winter and spring varieties and hard and soft cultivars. Other trichothecenes do occur concomitantly with DON, but DON is usually the most predominantly occurring toxin of the trichothecenes.

11. The presence of deoxynivalenol in small grains is associated with infections of the disease *Fusarium* head blight caused by *Fusarium graminearum* and *Fusarium culmorum*. *Fusarium* species can produce DON in the field and also during storage if the moisture content of grain kernels is high.

12. The occurrence of DON has been associated with Fusarium head blight infections, primarily at time of flowering, and, to a lesser extent, just prior to harvest and in the swath. Local temperatures, rainfall and humidity are major factors for infections that occur at the time of flowering. The timing of rainfall, rather than the amount, is the most critical factor. A relationship between the *Fusarium* head blight and contamination of wheat with deoxynivalenol has been established. Nevertheless, since not all species of the genus Fusarium produce DON, the occurrence of DON is not always quantitatively related to mould or *Fusarium* presence.

13. JECFA (ref 9) has assessed the levels and patterns of contamination of food commodities by deoxynivalenol on the basis of data received from Argentina, Brazil, Canada, China, Finland, Germany, Italy, the Netherlands, Norway, Sweden, the United Kingdom, Uruguay, and the USA and from data from the literature. Deoxynivalenol was found to be a frequent contaminant of cereal grains such as wheat (11444 samples, 57% positive), maize (5349 samples, 41% positive), oats (834 samples, 68% positive), barley (1662 samples, 59% positive), rye (295 samples, 49% positive) and rice (154 samples, 27% positive). It was also detected in buckwheat, popcorn, sorghum, triticale and in some processed food products such as wheat flour, bread, breakfast cereals, noodles, baby and infant foods, and cooked pancakes, and also in malt and beer. The mean concentrations in data sets in which samples containing deoxynivalenol were found were 4-9000 µg/kg for barley, 3-3700 µg/kg for maize, 4-760 µg/kg for oats, 6-5100 µg/kg for rice, 13-240 µg/kg for rye, and 1-5700 µg/kg for wheat.

14. The following data are an example of wide annual variation and show that DON is present in finished products. In the Netherlands about 1200 samples of wheat and wheat containing products were sampled and analysed for DON between 1998 and September 2001, as part of the monitoring programme of the Inspectorate for Health Protection. Samples included products such as wheat, breakfast cereals, bread, pasta, wheat flour, and many other foodstuffs. The data show higher DON levels in wheat products prepared from wheat harvested in the 'wet' year 1998, compared to products from wheat harvested in the 'dry' years 1999 and 2000. In wheat harvested in 1998 a mean level of DON of 446 µg/kg was found (n= 216), whereas in wheat harvested in 1999 and 200 the levels were 161 µg/kg (n=281) and 168 µg/kg (n=87) respectively. In bread, biscuit and crackers the mean level was found to be 220µg/kg in the 1998 harvest samples, and 118 µg/kg (17 samples) and 65 (µg/kg 22 samples) in the 1999 and 2000 harvest samples respectively. In baby and toddler food (mainly (whole wheat) breakfast cereals) the DON levels were 949 µg/kg (28 samples) in the 1998 harvest samples, and 71 µg/kg (16 samples) and 140 µg/kg (5 samples) in the 1999 and 2000 harvest samples respectively.

15. Not only mean levels are interesting, but also knowledge of the distribution and especially knowledge on the incidence of high concentrations is important to take measures. As an example, the percentages of samples of wheat containing more than 250, 500, 750, 1000 and 1250 µg/kg, respectively, were for the wet harvest year 1998 (n=158) 66%, 41%, 26%, 19% and 15%, respectively. The figures for 2000 (n=602) were 35%, 18%, 10%, 5% and 2%, respectively (ref 10).

16. Carry-over of deoxynivalenol to food products of animal origin does not appear of concern because animals refuse feed when the mycotoxin is present at high concentrations, and deoxynivalenol undergoes rapid metabolism and elimination in livestock species (JECFA 2001, ref 9).

17. The EC will undertake a scientific co-operation task to gather all available data on occurrence of DON and other *Fusarium* mycotoxins in foodstuffs in the EC and to make dietary intake estimates. It is expected that the first results of this study will become available by 31 December 2002 (ref 11).

Prevention

18. The 33rd session of the Codex Committee on Food Additives and Contaminants in 2001 agreed to return the draft Code of practice for the prevention of mycotoxin contamination in cereals, including annexes on ochratoxin A, zearalenone and fumonisins, to step 2 for redrafting by the delegations of the United States, in co-operation with Argentina, Canada, Norway, South Africa and Sweden. The Committee agreed that the code would include a new annex on trichothecenes. (ALINORM 01/12A para. 151, ref 1). Detailed information on prevention will be presented in document CX/FAC 02/21.

19. Fungicides and insecticides may influence the presence of DON, but fungicides are mainly developed to control pathogenic fungi and are rarely if ever specifically targeted to control toxigenic fungi. One should be careful not to selectively remove dominant pathogenic but non-toxigenic species, allowing more active colonisation by toxigenic species such as *Fusarium graminearum* (ref 12).

20. Research on prevention is progressing (ref 13). Identification of antagonists may result in patenting of biocontrol agents. Research is going on about the efficacy of anti-oxidants and essential oils.

21. A correlation has been established between the incidence and severity of *Fusarium* head blight infection and DON content of harvested grains. It was observed that the incidence and severity of *Fusarium* head blight were greatest when wheat followed corn and least when wheat followed non-cereals in a crop rotation system (ref 14). Agricultural practices such as crop rotation, plowing under or removing old seed heads, stalks and other debris will reduce the availability of crop residues in the soil and at the soil surface that can serve as nutrients for the saprophytic *Fusarium* species and hence will aid in the control of *Fusarium* head blight and DON contamination.

22. Another important means of preventing the contamination of wheat with DON is the use of wheat cultivars which are highly resistant to *Fusarium,* as well as reducing plant stress and appropiate drying after harvest.

23. The presence of DON cannot be completely avoided for the time being with the presently available prevention measures and techniques.

Decontamination and effects of processing

24. DON is considered to be a very stable compound, both during storage/milling and the processing/cooking of food. Therefore it can occur in foods prepared from DON-contaminated grains.

25. Post harvest safety management of wheat infected with Fusarium is an extremely important aspect of safety assurance. While strategic options are greatly limited by practical considerations, they involve developing ways to reduce, eliminate and control mycotoxin concentrations in commercial shipments and end-products.

26. Physical procedures for removing DON from contaminated grains, including cleaning, washing, dehulling, and milling have been successful to varying extents.The effectiveness of these procedures depend on the distribution of the toxin throughout the kernels as well as the level of contamination (ref 15).

27. It was found that when infected wheat was simply separated into fractions of various size with laboratory sieves, DON was concentrated in the smaller fractions. The larger fractions had low levels of DON (ref 16). Conventional wheat cleaning equipment has been used to separate *Fusarium*-damaged kernels with varying degrees of success (ref 17). Specific gravity tables also appear to be effective. It was found that most severely *Fusarium*-damaged kernels and associated DON were heavily concentrated in gravity table fractions of lowest density (ref 18). The most dense fractions had much less DON than corresponding unfractionated wheat. Removing the least dense fraction also improved the milling properties of the remaining wheat.

28. Wet-milling of corn is a major process for obtaining corn starch for human consumption. When DON contaminated corn was processed through a commercial wet-milling process, high levels of DON were found in concentrated steep liquor fractions, low levels in germ, fiber and gluten fractions, and very low levels(close to detection limits) found in the starch fraction (ref 19).

29. DON reduction during bread making in an Argentinian process occurs not only in the bakery due to thermal decomposition, but also during the fermentation step (ref 20). These baking results differ from other studies where DON survived the baking process.

30. DON survives the brewing process and has been found at high levels in beers from various countries (ref 21).

31. Research is ongoing to evaluate the efficacy of physical adsorbents for decontamination of DON-contaminated grain (ref 13). Other decontamination procedures are also investigated. No commercial methods are currently available for the complete removal of DON from contaminated grains.

Toxicology

32. JECFA performed a risk assessment of DON in 2001 (ref 9). Available toxicological data did not suggest that DON presents a carcinogenic hazard. In animals, decreased feed consumption, diarrhoea and vomiting have been observed as acute effects. JECFA recognised that deoxynivalenol can lead to outbreaks of acute illness in humans. However, the available data did not permit setting an acute reference dose (level below which no acute effects would be expected to occur).

33. Reduced growth and suppression of the host resistance to infection with *Listeria monocytogenes* and *Salmonella enteritidis* have been seen as short or long term effects. JECFA established a provisional maximum tolerable daily intake (PMTDI) of 1 µg/kg body weight and concluded that intake at this level would not result in effects of deoxynivalenol on the immune system, growth, or reproduction. As the trichothecenes have similar toxic properties, albeit with different potencies, JECFA recommended that toxic equivalency factors relative to DON be developed for the other trichothecenes commonly occurring in cereal grains, if sufficient data become available.

34. The European Scientific Committee on Food expressed its opinion on DON on 2 December 1999 (ref 22). The general toxicity and the immunotoxicity of DON are considered to be the critical effects. A temporary TDI (tTDI) of 1 µg/kg bw was derived based on a chronic dietary study with mice (safety factor of 100). The tTDI is made temporary because it is noted that DON belongs to the group of several trichothecenes with a common basic chemical structure which are produced by Fusarium fungi (e.g. T-2 toxin, HT-2 toxin, nivalenol). Furthermore, they may share common mechanisms of action. Once the most important trichothecenes have been evaluated, the Committee will consider the combined total exposure to trichothecenes and whether a group TDI should be assigned.

Exposure and risk characterisation

35. From the submitted occurrence data, JECFA estimated the dietary intake of deoxynivalenol on the basis of the single weighted mean concentrations for each commodity and the GEMS/Food regional diets. However, it should be noted that there was incomplete coverage for regions outside of the European region (the GEMS/Food European region includes North America), and that data from the European region was used to estimate concentrations in other regions. This use of European data could have lead to either an over- or under-estimate of exposure in regions other than the European region. The mean intake estimates (ranging from 0.77 to 2.4 µg/kg of body weight a day) values exceeded the PMTDI for four of the five regional diets. The Committee noted that there was considerable uncertainty in the intake estimates. However, the exceedance of the PMTDI by mean intakes in 4 of 5 regions suggests that it is quite likely that the PMTDI is exceeded by a substantial percentage of the world's population.

36. The European Scientific Committee in 1999 observed that the intake of DON from cereals and beer in the Scandinavian countries and from cereals in the Netherlands has been estimated to be in the order of the tTDI.

37. The Swiss Federal Office of Public Health estimated in 1997 the mean intake of DON in Switzerland: Adults: ≤ 170 ng/kg bw/day and young children: ≤ 800 ng/kg bw/day.

38. In the Netherlands, both the National Institute for Public Health and the Environment (RIVM) and the Dutch Health Council identified children as the population group most at risk to exceed the TDI (refs 10, 23). Of the 1-year old children, 80% exceeded the TDI and 20% exceeded 2xTDI. The intake of the 95 percentile of 1-year old children was 3 µg/kg bw. For both adults and children read is the most important food group contributing to the intake. For 1-year old children specific infant food also contributed significantly.

Maximum levels in food

39. The EC recently recommended an action level of 500 µg/kg for cereal foodstuffs as consumed and other cereal products at retail stage, and an action level of 750 µg/kg for flour used as raw material in food products. Such actions levels have been in use in *e.g.* the Netherlands since 2000. For some time, Austria has had a guideline level of 750 µg/kg for durum wheat and 500 µg/kg for wheat and rye.

40. Switzerland adopted a guideline level of 1000 µg/kg for DON in cereals in March 1998. This guideline level is valid for cereal products and for cereals as sold to the consumer, but does not apply to raw cereals.

41. Canada has a guideline level of 2000 µg/kg for DON in uncleaned soft wheat, corresponding to 1200 µg/kg in the flour portion (for the manufacture of non-staple foods such as cakes, cookies, biscuits). With respect to uncleaned soft wheat intended for use in infant foods, the guideline is 1000 µg/kg corresponding to 600 µg/kg in the flour portion. No guidelines have been developed for DON in hard wheat or other grains.

42. The US has a guideline value of 1000 µg/kg for finished wheat products.

43. Russia has a maximum level of 1000 µg/kg for DON in cereals (wheat of hard and strong types), flour and wheat bran (ref 24).

Maximum levels in feed

44. Maximum levels in feed are not needed to protect public health, but might be useful to protect animal health. Deoxynivalenol undergoes rapid metabolism and elimination in livestock species and is not known to occur at significant levels in foodstuffs of animal origin.

45. Belgium uses a guideline level of 5000 µg/kg for DON in cereals and cereal by-products used as raw materials for animal feedingstuffs. Austria recommended the following guideline levels: 500 µg/kg in feedingstuffs for pigs, 1,000 for µg/kg for feed for beef cattle and for feed for laying hens and breeding poultry, and 1,500 µg/kg for feed for fattening poultry. Germany has orientation values for DON of 1000 µg/kg for pig feed, 2,000 µg/kg for calve feed, 5,000 µg/kg for bovine and poultry feed. The following action levels are applied in the Netherlands: for CEREALS: for pigs, for laying hens, for calf and dairy cattle: 5,000 µg/kg, for other cattle and poultry: 10,000 µg/kg; for COMPOUND FEED: for pigs: 1,000 µg/kg, for calf and dairy cattle: 2,000 µg/kg, for laying hens: 3,000 µg/kg, for other cattle and poultry: 5,000 µg/kg. The US has the following guideline levels for DON in feed for various animal species: 10000 µg/kg for DON in grains and grain by-products destined for ruminating beef and feedlot cattle older than 4 months and for chickens, with the added recommendation that these ingredients not exceed 50% of the diet of cattle or chickens; 5000 µg/kg for DON in grains and grain by-products destined for swine with the added recommendation that these ingredients not exceed 20% of their diet; and 5000 µg/kg for DON in grains and grain by-products destined for all other animals with the added recommendation that these ingredients not exceed 40% of their diet. Canada recommends a maximum level of 5,000 µg/kg for DON in feedingstuffs for cattle and poultry and a maximum of 1,000 µg/kg for DON in feedingstuffs for swine and young calfs and lactating dairy animals (ref 24).

Trade disruption

46. In view of the worldwide contamination of cereals with DON, considering the fact that cereals are important in international trade and that different countries apply different rejection levels, it is expected that DON levels in commodities involved in international trade are a matter of concern.

47. Recall of foodstuffs because of elevated DON levels are reported, e.g. for pasta in Belgium and the Netherlands in 2001.

Other legitimate factors

48. Foodstuffs containing wheat are staple foods and are a good source of a number of nutrients. The Dutch Health Council therefore advises against reduction of exposure to DON by means of decreasing wheat consumption (ref 10).

Conclusions and recommendations

49. The appropriateness of setting of maximum levels of DON in foodstuffs derived from cereals should be discussed in CCFAC, as a result of the JECFA evaluation indicating that the TDI is exceeded in four of the five regional diets. Prevention of contamination is not sufficient, so that the setting and implementation of maximum levels should contribute to prevent the consumption of highly contaminated foodstuffs. When CCFAC chooses to set maximum levels for DON, as a second step CCFAC should determine the food groups for which maximum levels should be set, e.g. for (raw) cereals and cereal products and for infant foods. Finally, the levels need to be chosen based on the ALARA principle.

50. The appropriateness of setting maximum levels for DON in feedingstuffs derived from cereals should be discussed in CCFAC in view of ensuring the health and performance of animals.

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