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FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

WORLD HEALTH ORGANIZATION

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PROVISIONAL AGENDA

DISCUSSION PAPER ON DEOXYNIVALENOL (DON)

(prepared by the United States with the assistance of Belgium, Canada, European Community, Finland, France, Germany, Japan, Republic of Korea, the Netherlands, United Kingdom and the International Council of Grocery Manufacturers Associations)

Background

1. The 36th Session of the CCFAC agreed to discontinue consideration of maximum levels for deoxynivalenol (DON) for the time being. Instead, it agreed to request information on: the occurrence of DON in cereals; the influence of processing, decontamination, sorting, in lowering DON levels; national levels or guidelines for DON; and sampling procedures and methods of analysis, for consideration at its next Session (1).

2. The 37th Session of the CCFAC noted that more data on the occurrence of DON in cereals and processed cereal products were already available or would soon be made available on a more global basis. The Committee therefore decided to ask JECFA to conduct an exposure assessment based on the new data. In this regard, the Committee reconfirmed the importance to take into account processed foods and the effects of processing on the level of DON (2).

3. The Committee also decided to establish an electronic working group led by the United States to develop a discussion paper to provide comprehensive relevant data, including the occurrence of DON and the effects of processing on the levels of DON, for consideration at its next session. The members of the Working Group included: Belgium, Canada, European Community, Finland, France, Germany, Japan, Republic of Korea, Netherlands, United Kingdom and the International Council of Grocery Manufacturers Associations.

Introduction

4. Deoxynivalenol (DON; vomitoxin; “Rd-toxin”; 12,13-epoxy-3,7,15-trihydroxytrichothec-9-en-8-one ; CAS no. 51481-10-8), belongs to a class of sesquiterpenoid mycotoxns that are referred to as trichothecenes. The trichothecenes are produced by several fungi of the genus *Fusarium*, especially *F. graminearum* and *F. culmorum* which are pathogens of wheat, rye, barley, corn (maize) and other cereal grains. The worldwide distribution of the two species of fungi appears to be related to temperature; *F. graminearum* occurring predominately in warmer climates (3). The trichothecenes are the largest group of toxins produced by fungi of the genus *Fusarium* (4,5).

5. The trichothecenes are subdivided into four groups referred to as types A-D based on their molecular structure (6). Types A and B are the predominant ones widely distributed in cereals and feed as natural contaminants. Type A trichothecenes include T-2 toxin and HT-2 toxin, while type B trichothecenes include deoxynivalenol, nivalenol and their 3- and 15-acetylated derivatives. Type A trichothecenes are characterized by the presence of a saturated carbon at C-8, while type B trichothecenes have a carbonyl at the same position (7). Deoxynivalenol is found most frequently in cereal grains; however, other trichothecenes might co-occur with DON (8). Among the trichothecenes, type A members are more toxic than type B members (9).

6. *F. graminearum* and *F. culmorum* are found world-wide in soil and are responsible for the *Fusarium* head blight disease (FHB) in cereals resulting in the production of DON. Studies have shown that the severity of *Fusarium* head blight depends mainly on climatic effects (temperature, rainfall, humidity) (10). *F. graminearum* is also mainly responsible for Gibberella ear rot (red ear rot) disease in corn resulting in the production of DON. These fungi usually infect susceptible grain crops in the field when there is cool wet weather at the silking or anthesis stage of grain development (11). The incidence and severity of DON contamination is known to vary from region to region, from one season to the next, and also in different cereal species (12,13). These variations may be associated with periods of heavy rainfall between the anthesis stage and the time of harvest; this can facilitate *F. graminearum* infections and accumulation of DON (14). The infection of cereal grains by these fungi can result in the production of a mixture of several structurally related trichothecenes in addition to deoxynivalenol; the individual trichothecenes making up the mixture may vary by country as well as by region. DON is water-soluble, very stable during storage and milling, relatively heat stable, shown to survive most processing and cooking procedures, and is not completely destroyed by fermentation (15,16).

Toxicology

7. The International Agency for Research on Cancer (IARC) reviewed the toxicity of DON and some other toxins derived from *Fusarium graminearum* and *Fusarium culmorum* in 1993 (17). IARC concluded that there is *inadequate evidence* in humans for the carcinogenicity of toxins derived from *Fusarium graminearum*, *inadequate evidence* in experimental animals for the carcinogenicity of DON and that the toxins derived from *F. graminearum* and *F. culmorum* were not classifiable as to their carcinogenicity to humans (Group 3).

8. Risk assessments and toxicological reviews for DON have been conducted by the Nordic Council of Ministers, the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the European Commission's Scientific Committee on Food (SCF) the European Scientific Panel on Contaminants in the Food chain and other investigators (18-22). A provisional maximum tolerable daily intake (PMTDI) of 1 µg DON/kg bw was established by JECFA. Important toxicity data relevant for the characterization of hazards associated with trichothecenes was recently summarized (23,24).

9. Exposure of some domestic animals to DON results in loss of appetite, feed refusal and vomiting, accompanied by a decrease in weight gain (21). Many feeding studies have been conducted on swine because this species appears to be more sensitive to DON than poultry, ruminants and other livestock. Reduced feed consumption and lower weight gain have been reported as the principal clinical effects observed in swine following ingestion of low dietary levels (< 2ppm feed) of DON in naturally contaminated feedstuffs; higher doses may induce vomiting and complete feed refusal (25). In several studies involving pigs, where the toxicological responses in animals that were given feed spiked with a known quantity of pure DON were compared with responses noted in animals given naturally contaminated feed containing an equivalent quantity of DON, it was observed that naturally contaminated feed had a stronger effect on the feed intake and weight gain parameters than the pure toxin (21). It is possible that immunosuppression, hematological and histological changes seen in animals fed naturally contaminated feed containing DON may be intensified by co-contamination with other mycotoxins. It is unknown why swine are more sensitive to DON than other animals. Toxicological evaluations of the fate of trichothecenes in laboratory animals as well as other animal species, have been published (19, 26, 27). A major conclusion from those evaluations was that trichothecenes are rapidly excreted from animals, and therefore there is no significant carry-over of these toxins from animal derived products to humans.

10. Human poisonings associated with the consumption of trichothecene- contaminated grains (wheat, barley, corn) have been reported in Korea, Japan, India, Colombia, China and South Africa (15,24,28,29). Symptoms including nausea, vomiting, diarrhea, abdominal pain, headache and dizziness were observed. The precise role of DON in those outbreaks is not certain. Although relatively high levels of DON were detected in some samples of moldy corn and scabby wheat and *Fusarium* species were identified, a rigorous correlation could not be demonstrated between DON and the human symptoms (29). The epidemiological data available however, point toward trichothecenes or specifically DON-contaminated grain products as a potential causative factor of the acute human toxicosis.

11. A review of the toxicity of DON and its potential effects on humans was recently published **(**24). A urinary biomarker that can be used for DON in both humans and animals was recently developed; the use of this biomarker should facilitate epidemiological studies of the adverse toxicological effects associated with this mycotoxin (30). The co-occurrence of several *Fusarium* toxins, along with zearalenone, has been observed in cereal grains. The presence of other toxins in grains is of concern because of the possible interactions of these toxins and their combined impact on the toxicological responses observed in animals and humans (20,31,32).

Sampling

12. It is difficult to estimate accurately and precisely the mycotoxin concentration in a large lot of grain because of the large variability associated with the mycotoxin test procedure. A mycotoxin test procedure generally consists of 3 steps: (a) an aggregate sample (consisting of incremental samples taken from various locations within a lot) is taken from the lot, (b) the whole aggregate sample is finely ground in a mill to reduce particle size, and a subsample is removed from the comminuted sample for extraction by solvents, and (c) the mycotoxin is extracted from the comminuted subsample and quantified by analytical techniques (33). The variance associated with the mycotoxin test procedure that measures DON in cereals is the sum of sampling, sample preparation, and analytical variances (34).

13. Statistical studies (34) have shown that the coefficients of variation associated with testing wheat are relatively small compared with testing other commodities for mycotoxins, such as aflatoxins in peanuts. The smaller variability (relative to that for other mycotoxins and other food commodities) is due in part to the kernel count of wheat per unit mass (about 30 kernels per gram), which is about 10 times larger than that for shelled corn and 30 times larger than that for shelled peanuts, however, other factors are also involved. This suggests that the distribution of DON contamination among wheat kernels may be less skewed than for aflatoxin in other commodities.

14. The variability associated with a mycotoxin test procedure can be reduced by increasing sample size, the degree of sample comminution, subsample size, and the number of aliquots quantified (33). A knowledge of the variability associated with sampling procedures involving cereal grains, when coupled with the availability of validated analytical methods and appropriate information on the distribution and tolerance or guideline levels for DON, can be used to: (1) provide an estimation of errors in the evaluation of DON concentration in lots of cereal products, (2) design sampling plans for DON in cereals and (3) select sample size or number of samples needed to reduce the total variability of an entire test procedure.

Analytical

15. Many analytical methods have been developed for the detection and quantification of DON and other trichothecenes. These toxins can be separated and analyzed by thin-layer chromatography, various liquid, gas and supercritical fluid chromatographic procedures, as well as by immunochemical methods. There are comprehensive reviews of current methodologies that can be used for the detection and analysis of DON and other trichothecenes in cereals (35-45).

16. Thin-layer chromatographic procedures for the detection and analysis of DON are reliable and cost-effective for use in laboratories with a restricted budget. Liquid chromatographic procedures alone or coupled with mass spectrometry and various detectors are becoming more common in many laboratories for the analysis of DON and other type B- and A- trichothecenes. Gas chromatographic procedures can also be used for the determination of many trichothecenes. The toxins or their derivatives are mostly detected by flame ionization, electron-capture detectors or by coupling to a mass spectrometer. Whenever possible and affordable, it is recommended that thin-layer chromatographic methods for the determination of DON be replaced gradually by advanced chromatographic techniques, ideally coupled to a mass spectrometric detector.

17. Immunochemical methods have received a lot of attention lately because they can be used for rapid screening purposes under field conditions or in the laboratory and some can also effectively complement gas and liquid chromatographic procedures that are widely used in routine monitoring. These methods are simpler and less labor intensive. Comprehensive reviews of immunochemical and other rapid methods that can be used for trichothecenes have been published (37, 46-47). Information on mycotoxin test kit data bases can be obtained from the homepages of AOAC International (www.aoac.org) and on the European Mycotoxin Awareness Network (EMAN) (www.mycotoxins.org).

18. Quantitative analytical methods that are to be used for monitoring and enforcement purposes should be validated or collaboratively studied to ensure that the analytical results obtained give an accurate measure of the analyte involved. Methods that have been developed and validated for the extraction and detection of DON in whole grain kernels cannot effectively be used for processed cereal grain products without further modifications in most cases. In conducting surveys involving milled or processed cereal products, it is essential that recovery studies be done for each type of product analyzed in order to determine if changes in DON levels represent accurate levels of the toxin or represent poor recovery of the toxin from the product matrix. Certified reference materials (CRMs) are available for DON in wheat and maize and these can be used to demonstrate that the method used provides accurate results (37,48). Two commercially available crystalline DON analytical standards used by some laboratories throughout the world have been determined to be >96 and >98% pure, respectively (49).

19. Current AOAC official methods of analysis for DON in wheat include thin-layer chromatography (method 986.17) and gas chromatography (method 986.18). A liquid chromatographic method for determination of DON in whole wheat flour, white flour and bran has been subjected to an interlaboratory study and adopted as a peer-verified method by AOAC International (50). An international interlaboratory study has been conducted to compare different methods for DON analysis (37, 51). Due to the natural co-occurrence of DON with other trichothecenes and zearalenone, emphasis should be placed on the development and validation of methods capable of detecting multi-mycotoxin residues. Liquid chromatography/ tandem mass spectrometry methods have recently been made available for the rapid and simultaneous determination of zearalenone as well as trichothecenes (52,53).

DON in cereal grains

20. The world-wide occurrence of DON in cereal grains has been well documented and reviewed in the literature (9,15,54,55). Wheat, barley and corn together account for two-thirds of the world production of cereals and are the crops most susceptible to *Fusarium* disease and trichothecene contamination (56). DON has been found in other cereal grains including rye, oats, rice, and their products in many regions of the world (9). Studies have shown that at low levels of contamination, DON and its acetylated derivatives are produced and remain localized in the outer parts of the grain kernels to a large extent (57). However, at higher levels of contamination, the toxins may be more evenly distributed throughout the kernel (58). Zearalenone is known to co-occur with DON and other trichothecenes since it is produced by the same *Fusarium* species. A “masked” naturally occurring glucoside of DON (DON-3-glucoside) was recently found in *Fusarium*-infected wheat and maize (59). The identity of the compound was confirmed by NMR and its presence in naturally contaminated maize and wheat ranged from 4 to 12% of the DON concentration.

21. Analytical data on DON, resulting from surveys conducted on cereals and cereal products in many parts of the world between 1990 and 2000, have been summarized (60). DON was investigated in 15,187 samples; 48.1% were from wheat (mean 531 µg/kg, 61.6% positives), 20% from corn (mean 230 µg/kg, 60.3% positives) and 18.5% from wheat products (mean 314 µg/kg, 52.2% positives). DON was also detected in other cereals such as barley, oats, rye, rice and in processed food products such as beer, cooked pancakes, baby and infant foods, breakfast and mixed grain cereals, noodles and cookies at lower levels.

22. In a recent survey conducted by the EU involving 12 member countries, a total of 11,022 samples of various food and food raw materials were analyzed for DON, nivalenol, T-2 toxin and HT-2 toxin (61). Fifty-seven percent of the samples were positive for DON and wheat and wheat products represented the largest number of the positive samples. Among cereals, corn showed the highest level of contamination with trichothecenes. Seven percent of the raw cereals and flour contained DON levels at 750 µg/kg or higher and 6% of the cereal products contained DON levels at 500 µg/kg or higher. Wheat and wheat containing products such as pasta and bread represented the major sources of intake of the four trichothecenes that were measured.

23. In a survey of stored grain from the 1999 harvest in the UK, DON was detected in 88% of the 320 samples of wheat, barley and oats analyzed; 83% were below 100 µg/kg; the maximum level was 600 µg/kg. In samples with DON levels exceeding 150 µg/kg, nivalenol was also detected at 50 µg/kg or higher (62).

24. In Croatia, 465 samples of feed grain were collected and analyzed over a 7 year period from manufacturers and farm storage facilities (63). DON was detected at levels ranging from 50 to 340 µg/kg in 41.2 % of the samples. The majority of the samples were for poultry feed.

25. Two hundred and seventy-two oat samples were analyzed after harvest in an area of southwest Germany over a five year period (64). DON was the major toxin with incidences at 49-85% and mean levels in positive samples of 52-302 µg/kg. A correlation was noted between the occurrence and levels of the trichothecenes in oats and the amount of heavy precipitation during the summer months preceding crop harvest.

26. A survey for DON in cereal grains harvested over a 3 year period was conducted in Russia (65). DON was detected in 69% of 2,166 stored wheat samples from the major *Fusarium* epidemic region of Russia. The contamination levels ranged from 100 to 8,600 µg/kg and a positive correlation was noted between DON concentration and the percentage of *Fusarium*-damaged wheat kernels. DON was detected in 11% of 1,908 freshly harvested food wheat samples; the concentration levels of DON ranged from 50 to 6,650 µg/kg. The incidence and levels of DON in freshly harvested barley and rye were significantly lower than in wheat.

27. A total of 843 commercial animal feed and foodstuffs samples were collected and analyzed for trichothecenes over a 3-year period in Saudi Arabia (66). DON was the most frequent trichothecene detected (13% of all the positive samples examined) and the levels ranged from <2 to 4,000 µg/kg. It was present in 21% of the maize samples and in 18% of the poultry feed, but the highest concentration was recorded in barley samples where it had a mean concentration of 2,553 µg/kg.

28. Two thousand five hundred and twenty-four wheat samples were collected and analyzed for DON in the U.S from 1994 to 2003 (67). Forty-one percent contained DON at levels less than 500 µg/kg; 18.6 % contained >500 to 1,000 µg/kg; 39.8% contained >1,000 to 6,000 µg/kg and 0.6 % contained > 6,000 µg/kg. DON levels varied significantly from year to year.

29. Two thousand one hundred and six barley samples were collected and analyzed for DON in the U.S. from 1993 to 2003 (67). Thirty-eight percent contained DON levels less than 490 µg/kg; 14.5 % contained 490 to 990 µg/kg; 28.5 % contained >990 to 4,990 µg/kg and 18.6% contained 4,990 to >5,000 µg/kg. As for wheat, year to year variation was noted in DON levels.

30. During the first three years (2001-2004) of a five year project in the UK to look at agronomic factors affecting the production of *Fusarium* toxins, including DON, 1,200 wheat samples were analyzed (68). Ninety-seven percent contained less than the EU limit of 1,250 µg/kg DON. Location was a major but not always a consistent factor, from year to year. The risk of DON contamination was high when a wheat crop followed a maize crop. No difference was noted between DON levels in wheat samples obtained from organic and conventional crops.

31. Two thousand, nine hundred and twenty-four wheat samples were analyzed for DON in the Netherlands from 1998-2004 (69). The mean DON content of the wheat was 580 µg/kg, in the year 1998, which was the year with the highest *Fusarium* contamination. In the other years, the average DON levels ranged between 190 and 317 µg/kg. The effect of different DON limits on the mean DON content of wheat lots approved for sale to the milling industries was also calculated.

32. In Japan, 136 samples of husked wheat were examined in 2001 and 2002 (70). Seventy- seven percent of the samples analyzed in 2001 contained an average DON level of 286 µg/kg. Ninety-five percent of the samples analyzed in 2002 contained an average DON level of 184 µg/kg. Additionally, six-hundred and thirty-eight samples of husked wheat (domestically produced) were analyzed in 2002-2004; 37% contained DON with maximum levels of 2,100 (2002), 580 (2003) and 930 (2004) µg/kg. The 90th percentile levels were 570 µg/kg (2002), 260 µg/kg (2003) and 140 µg/kg (2004).

Studies to reduce DON levels in grains

33. Various physical procedures including cleaning, washing, sieving, density segregation, dehulling, fractionation by specific gravity table and polishing have been used singly, or in combination with milling procedures to reduce the level of DON in grains. The effectiveness of these procedures depended on the extent of contamination and the distribution of the toxin throughout the grain (71,72). Milling procedures to remove DON from wheat and other grains rely substantially on physical separation of the more heavily contaminated outer layers of the grain kernels.

34. Dry-milling is a process by which the components of cereal grains are separated into fractions based on particle size. The different fractions, such as flour and meal, retain most of the characteristics of the original grain (16). Studies have shown that in milled wheat, higher concentrations of trichothecenes are found in the bran fraction than in the original wheat with lower concentrations in white flour (72-75). Flour processing techniques can reduce DON levels by approximately a factor of 2 or greater. The extent to which dry-milling can reduce the DON level in flour is dependent on the extent of the fungal penetration into the endosperm of the wheat kernel and the distribution of DON within the kernels. The extent of fungal penetration is dependent on the cultivar of wheat involved (76,77). Dry-milling of DON contaminated corn results in the DON being concentrated in the germ meal fraction (78).

35. Wet-milling is a process widely used for corn to obtain starch which can be used for the production of syrups and other products for human consumption. Since DON is highly soluble in water it is partitioned into the aqueous phase during the wet-milling process with negligible accumulation in the solid residue used for food products (16,79).

36. Many liquid and gaseous chemicals have been tested for their effectiveness in reducing DON levels in contaminated grain. Most of the chemicals tested resulted in little or no significant reduction in the levels of DON (71,72). Sodium bisulfite was found to reduce DON levels in corn but it cannot be used directly for human food because it affects the rheological properties of flour and the DON adduct formed is not stable and hydrolyzes back to DON under certain processing conditions (76).

37. Ionizing radiation, extrusion and thermal processing procedures have been developed that can reduce DON levels to some extent under very specific conditions. However, there is no single method currently available that can completely remove all DON from cereals (71,80).

38. The Ministry of Agriculture, Forestry and Fisheries of Japan has been funding research for developing color sorting machines and pearling machines for wheat grains (70). Results from preliminary experiments show that prototypes of these machines may be helpful in separating DON contaminated wheat kernels from non-contaminated kernels.

DON in processed products

39. The process of converting raw and milled grains into food for human consumption has significant effects on the levels of DON in the finished products. Humans are exposed to DON contamination mainly as a result of contamination of finished products. DON is a relatively heat stable molecule; it is stable at 120^{0}C, moderately stable at 180^{0}C and partially stable at 210^{0}C. DON is water-soluble and stable under weakly acid conditions but not stable in alkali (19).

40. A total of 190 samples of common wheat, durum wheat and rye flours were collected from mills and the retail markets in Denmark between 1998 and 2001 and analyzed for DON and nivalenol (13). DON had an incidence rate of 78% over all samples for all years. The contamination level varied from year to year. The highest incidence and DON levels were found in wheat and rye samples from the 1998 harvest and this was attributed to the unusually cool and wet growing season that year. The mean concentrations in the wheat and rye flours were 191 µg/kg and 99 µg/kg, respectively. DON was found in approximately 50% of the rye samples collected between 1998 and 2000, with a mean concentration of 49 µg/kg. Durum wheat flour showed the highest DON contamination level and all samples collected during 2000 and 2001 contained DON with means and medians above 100 µg/kg. Over 70% of the samples contained more than 500 µg/kg DON and the highest observed concentration was 2,591 µg/kg.

41. Of 60 wheat samples analyzed in Argentina for DON, 93.3% were contaminated, and the average level was 1,798 µg/kg (81). Sixty-one samples of wheat flour were analyzed and the average DON level was 1309 µg/kg; the average DON level in 42 different bakery products was 464 µg/kg. This survey was done on samples from the 1993/94 wheat crop which experienced a rainy season.

42. During the years 2001-2004, a total of 4,965 food samples, purchased from the German market, were analyzed for DON in Germany (82). DON was found in most foods containing cereals with an incidence of greater than 50%. For foods such as breads, rolls, and pasta, the incidence of DON contamination was typically 70-90%. The highest DON contamination was found in durum wheat and products thereof. Median DON levels were about 2-10 times higher than those of other frequently contaminated cereals (soft wheat, maize and products thereof) with maximum DON levels of 2,000-3,000 µg/kg. The mean and median average levels for DON in most products, with few exceptions, were well below the current maximum permitted levels in Germany (100-500 µg/kg). Relatively minor qualitative and quantitative differences were noted in the contamination of foods with DON between years. Regional differences were not noted, although incidence and levels were much lower in products resulting from organic farming than in those from conventional production.

43. A total of 562 wheat-based products from the 1993 crop year in the United States were collected and analyzed (83). The percent of samples with DON contamination greater than 1,000 µg/kg in bran, white flour, whole wheat flour, and miscellaneous test samples were 12, 10, 16 and 5, respectively. About 52, 50, 40 and 27% of the same test samples were contaminated with DON at levels > 100 µg/kg. The 1993 wheat crop in the Midwestern part of the United States experienced cool, wet conditions during the spring and summer months thus resulting in elevated levels of DON in the crop harvested that year.

44. A total of 728 wheat-based products were examined in the United States between 2000 and 2004 (67). The percent of samples with DON contamination greater than 1,000 µg/kg in wheat bran, wheat flour and other milled wheat products were 17.5, 1.0 and 1.8, respectively. The percent of the same test samples containing greater than 100 µg/kg were 31, 37 and 36, respectively.

45. Adult cereal-based breakfast foods from the Canadian retail market were surveyed for several mycotoxins over a 3-year period beginning in 1999/2000. Depending on the year, DON was detected in 40 to 59% of samples with mean levels ranging from 10 to 70 µg/kg (84).

46. Cereal-based infant foods from the Canadian retail market were surveyed for several mycotoxins over a 3-year period. DON was detected in 63% of the samples, with mean levels ranging from 32 -150 µg/kg (85). In a similar survey of cereal-based infant and baby foods in southwest Germany, DON was detected in 60% of the samples with DON levels ranging from 15 -314 µg/kg (86).

47. A survey of 101 commercially available breads on the German market in 1999, revealed incidence levels of DON , nivalenol and 3-acetyldeoxynivalenol of 92, 5 and 8%, respectively; the median levels in positive samples were 134, 25 and 40 µg/kg, respectively (87). DON levels were lower in bread samples produced from cereals organically grown compared to cereals conventionally grown, however, there are many factors that may contribute to the differences observed between organically and conventionally grown cereals and their milled and finished products (88).

48. Two hundred and nineteen samples of grain-based food, pseudocereals and gluten-free food were collected from food and health food stores in Germany (89). These samples were analyzed for 13 trichothecene toxins including DON, 3-acetyl DON, 15-acetyl DON and nivalenol; the incidences of contamination were 57, 1, 13, and 10% respectively. The highest level of DON was 389 µg/kg while the levels of the other toxins were less than 100 µg/kg.

49. Sixty samples of white and whole grain wheat flour were collected during 1999 from mills and food stores in an area of southwest Germany (57). DON was the predominant toxin found. Based on total samples, the incidence of DON, nivalenol, 3-acetyl DON, 15-acetyl DON, HT-2, T-2 and zearalenone were 98, 12, 2, 3, 7, 2 and 38%, respectively; the median levels of the positive samples were 199, 25, 11, 15, 12, 4 and 3 µg/kg, respectively.

50. Three hundred and thirty-five retail oats samples were collected and analyzed over a 6 month period in 2003 by the Food Standards Agency in the UK (90). A total of 6 trichothecenes were detected at levels ranging from 10 to 404 µg/kg in 52% of the samples; DON, T-2 and HT-2 toxins were among the most common.

51. A survey consisting of 377 retail cereal samples were analyzed for a number of trichothecenes in the UK during 2003 (91). Two hundred and ninety-eight samples contained detectable toxins; DON and nivalenol were the most frequently occurring. The highest levels of DON occurred mainly in maize-based breakfast cereals and snacks, at 2,261 and 879 µg/kg, respectively.

52. During 2002-2003, 164 samples of wheat flour were analyzed in Japan (70). Eighty-five percent of the samples analyzed in 2002 contained DON with an average level of 138 µg/kg. Seventy-four percent of the 2003 samples contained DON; the average level was 43 µg/kg. The reduction of the DON level in flour samples during 2003 was attributed to the provisional maximum level for DON in husked wheat that was established at 1.1 mg/kg by the government of Japan in May 2002.

53. The controlling regulatory agencies in The Netherlands analyzed samples of cereals and cereal products for DON during the years 2001 to 2004 (69). Of 447 samples of unprocessed cereals analyzed, 19% contained DON levels less than100 µg/kg, 52% contained levels between 100 and 750 µg/kg, 2% were between 750 and 1000 µg/kg and 3% were above 1000 µg/kg. Two hundred and thirty-nine samples of self rising flour were analyzed for DON; 91% had DON levels less than 100 µg/kg and 9% had levels less than 750 µg/kg. Four hundred and forty-seven pasta samples were also analyzed for DON; 95% of the samples contained less than 500µg/kg of DON, 4% contained between 500 and 750 µg/kg and 3% contained greater than 750 µg/kg DON. The co-occurrence of ochratoxin A was noted in some samples of the flour and unprocessed cereals

Studies associated with the reduction of DON levels in processed foods

54. Comprehensive reviews on the influence and effectiveness of various processing procedures used to reduce the DON levels in milled cereal grain products, beer and finished bakery products have been published (71,91-93).

55. Research efforts in many countries are currently focusing on possible ways of reducing the DON levels in finished food products that are prepared from raw cereals that may be contaminated with DON. In studies of this nature, it is important that naturally contaminated (field infected) cereal grains be used as the starting material because the pattern of *Fusarium* infection in the kernels is crucial to the subsequent fate of the toxins during processing (91).

56. In cooperation with the cereal industry in the UK, work is being carried out to measure the levels of *Fusarium* mycotoxins including DON in raw cereals (wheat, maize and oats) and then determine how the key stages of food processing affect the contamination of the final food product (68). The research is aimed at determining the factors that affect toxin levels at each process stage by using laboratory and pilot scale studies and sampling from production plants. The knowledge generated from this project is intended to assist industry to further reduce mycotoxin contamination. This work is also considering the formation of metabolites and bound residues and any toxicological implications arising from those.

57. A recent study was conducted in Argentina to determine the distribution of DON in various fractions resulting from the milling of naturally contaminated wheat using an industrial milling process (75). The levels of DON in the raw wheat, flour, bran and gluten were 1,928, 994, 4,680 and 293 µg/kg, respectively.

58. The stability of DON in wheat flour was evaluated during the fermentation stage of bread-making on a pilot scale in Argentina (94). Using flour containing 150 µg/kg of DON, fermenting the dough at 50^0C resulted in the DON level being reduced by 56% for Vienna bread and 49 % for French bread. The investigators concluded that the DON reduction during bread making might be due to some process that occurs during yeast fermentation and not solely to thermal decomposition.

59. A detailed study to determine the influence of *Fusarium* infection of wheat on the baking quality of wheat, was recently conducted in Germany (95). The results indicated that high *Fusarium* infection levels accompanied by high DON levels did not necessarily deteriorate the baking quality of DON contaminated wheat.

60. A study was initiated to determine the extent of reduction in levels of DON that might occur during various steps in the processing of unclean naturally contaminated durum wheat to the production of cooked spaghetti (12). With respect to the unclean wheat, the average levels of DON decreased to 77% in cleaned wheat, 37% in semolina, 33% in raw spaghetti and 20% in the cooked spaghetti. Average DON levels in the screenings, bran and fine middlings were 4.1, 1.6 and 0.6 fold respectively, relative to the unclean wheat. The results from this study conservatively suggest that the cooked pasta retains 25% or less of the level of DON found in grains.

61. The retention of DON in processes involving milling and finished food production using naturally contaminated wheat was studied recently in Japan (96). The milled flour retained 40-55% of the original concentration of DON in the raw wheat and almost 200% of the DON concentration was found in the bran fraction. When the flour was baked into bread, DON was retained at the same level as in the dough. During processing of Japanese-style wheat noodles, only 30% of DON in the dough was detected in the cooking noodles, the cooking water solids retained 44.7% of the DON levels. The overall reduction of DON contamination level from wheat to finished product was conservatively estimated to be 51% for bread and 14% for noodles.

Regulatory status

62. At least 37 countries have established regulatory limits or guidance levels for DON in foods and feeds (97). The guideline levels for cereal and finished cereal products intended for human consumption ranged from 100 µg/kg to 2,000 µg/kg. The guidance levels for DON in the diets of swine, poultry and cattle range from 500 µg/kg to 10,000 µg/kg depending on the age of the animal species.

Risk Management (Control)

63. There is no single practical method available for significantly reducing DON contamination levels in wheat and other cereal grains at this time. Various physical and chemical procedures have been effective in reducing DON contamination in harvested cereal grains to varying extent. However, current technology cannot prevent cereal crops from becoming contaminated with DON and related trichothecenes before harvest. The incidence and levels of DON in cereal crops throughout the world vary considerably depending on many factors including environmental conditions, cultivar of cereal planted, and traditional agronomic practices employed in different countries. In order to manage the risk associated with DON contamination in cereals, an integrated risk management system approach is needed. This includes pre-harvest management (including Good Agricultural Practices), harvesting management (to include time of harvest, control of temperature and moisture during transportation and storage of grains) and post-harvest management (including Good Manufacturing Practices, decontamination and diversion strategies) with appropriate controls at each level (98). Before long-term risk management decisions can be made internationally, more information is needed on the year-to-year variability of the levels of DON in cereals grown in many parts of the world as well as the consumption patterns of various populations.

64. In 2003, the Codex Alimentarius Commission (CAC) adopted a *Code of Practice for The Prevention and Reduction of Mycotoxin Contamination in Cereals, Including Annexes on Ochratoxin A, Zearalenone, Fumonisins and Trichothecenes.* (CAC/RCP 51-2003). The implementation of the practices pointed out in that document, along with advances in post-harvest techniques proper drying and storage conditions followed by good manufacturing practices (GMPs), may substantially reduce the levels of DON in the food supply.

65. Comprehensive reviews of cropping systems and pre-and post-harvesting agricultural practices that may reduce or prevent *Fusarium* contamination of cereal crops have recently been published in the scientific literature (10, 99-101).

Conclusions and Recommendations

66. Codex member states should be encouraged to continue to submit data from surveys of levels of DON in cereal products in their countries, using validated analytical methods, and over a period of several years to reflect seasonal variations. These data would be used, taking into account regional differences in food consumption patterns, to determine exposure estimates and for use in developing an appropriate international standard for DON in wheat.

67. Research involving the breeding of cereal cultivars (wheat specifically) that are resistant to the growth of *F. graminearum* and *F. culmorum* and the resulting *Fusarium* head blight disease that can develop in wheat, should be encouraged as well as strategies that can be implemented to help prevent the production of trichothecenes in cereal grains.

68. Research on methods to prevent and/or reduce contamination of cereal grains in the field, during storage and processing by *Fusarium* species should be encouraged and continued. There is need for a better understanding of the *Fusarium*-grain interactions in symptomatic and asymptomatic infections of grains in the field. Studies are needed to identify and determine the toxicity of products resulting from the degradation and chemical modification of DON and other trichothecenes as a result of various processing procedures.

69. CCFAC should defer development of international standards until more regional data on incidences and levels of DON in cereals over a period of several years is available. In addition, adequate information for determining consumption patterns for various countries is needed.

70. The toxicity of the 3-acetyl and the 15-acetyl DON that occur along with DON need to be investigated with respect to their contribution to overall DON toxicity since they often occur at levels of 10-20% of the level of DON.

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