codex alimentarius commission

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

WORLD HEALTH ORGANIZATION

JOINT OFFICE: Via delle Terme di Caracalla 00100 ROME Tel.: 39.06.57051 Telex: 625852-625853 FAO I E-mail: Codex@fao.org Facsimile: 39.06.5705.4593

Agenda Item 16(g) **CX/FAC 00/22**

January 2000

JOINT FAO/WHO FOOD STANDARDS PROGRAMME

CODEX COMMITTEE ON FOOD ADDITIVES AND CONTAMINANTS
Thirty-second Session
Beijing, People's Republic of China, 20-24 March 2000

POSITION PAPER ON FUMONISINS

INTRODUCTION

1. The 31st Session of the Codex Committee on Food Additives and Contaminants (CCFAC) accepted the offer of the United States to develop a Position Paper on Fumonisins (ALINORM 99/12A, para. 97).

2. Fumonisins are a class of recently identified mycotoxins that are produced mainly by *Fusarium moniliforme [= F. verticillioides], Fusarium proliferatum* and several other *Fusarium* species. *F. moniliforme* is a fungal pathogen of corn (*Zea mays*) and is one of the most prevalent fungal species associated with corn throughout the world (1,2).

3. Fumonisins isolated from cultured strains of *F. moniliforme* have produced neurotoxicity in horses, adverse pulmonary effects in swine and induced carcinogenisis in the livers of rats (3). There is also suggestive evidence from limited epidemiological studies that fumonisins may be carcinogenic in humans (e.g., human esophageal cancer). Fumonisins have been detected in corn and corn products designated for human consumption.

4. Fumonisins are a structurally related group of diesters of propane-1, 2, 3-tricarboxylic acid and various 2-amino-12, 16-dimethylpolyhydroxyeicosanes in which the C14 and C15 hydroxyl groups are esterified with the terminal carboxyl group of tricarboxylic acid (4). There are at least 12 fumonisin analogues that have been identified and these have been classified into series A, B, F and P based on their chemical structure (5). The B series, consisting mainly of fumonisin B1 (FB1), and fumonisin B2 (FB2), are believed to be the most abundant and most toxic naturally occurring analogues (6,7). The extent of contamination of corn with fumonisins varies with geographical location, agricultural practices, and the corn genotype which determines the susceptibility of the corn plants to fungal and insect invasion during the growing phase of the corn in the field.

5. *F. moniliforme* is a soil-borne as well as a seed-borne pathogen of corn, therefore the extent of infection of the corn plant and kernel by the fungus will vary depending on the point, or points, of entry into the developing corn plant (8,9). The levels of fumonisins produced in corn are influenced by environmental factors such as temperature, humidity, drought stress and the extent of rainfall during the preharvest and harvesting periods; storage of the harvested corn kernels under improper moisture conditions can result in additional accumulation of fumonisins (8). Surveys of corn grown in many countries around the world have revealed the presence of varying levels of fumonisins; a significant percentage of apparently healthy-looking corn kernels contained fumonisin levels of about 1 μg/g (ppm) or higher (10,11). Higher levels of fumonisins are usually found in corn kernels produced in the warmer regions of the world (12,13). The ratio of FB1/FB2 is approximately 3:1 in naturally contaminated corn (14).

TOXICOLOGICAL EVALUATIONS

6. The International Agency for Research on Cancer (IARC) reviewed the toxicity of *Fusarium moniliforme* derived toxins in 1993 (15). IARC classified the toxins derived from *F. moniliforme* as possible human carcinogens (Group 2B). Fumonisins will be evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in the near future (February,2001).

7. There is no direct scientific evidence that fumonisins cause adverse effects in humans. Studies currently available demonstrate inconclusive associations of fumonisins with human disease. Investigators in South Africa have noted a correlation between high levels of fumonisin-producing molds on corn used to make alcoholic beverages and esophageal cancer in human subgroups (16). Those studies were limited by the lack of controlled conditions, particularly for established confounding risk factors (e.g.,alcohol consumption) and therefore do not allow any definitive conclusions to be made about cancer causation in humans. Other studies lacked similar controls or did not measure fumonisin levels (17,18). In a recent limited epidemiological study in India, an association of high levels of fumonisins (but not other mycotoxins) in moldy sorghum and corn with mild gastrointestional symptoms was noted (19). However, this study also suffered from a lack of control of established risk factors. In addition, contaminants other than mycotoxins cannot be eliminated as causative factors, and a similar association was not detected in studies conducted in other countries.

8. Ingestion of fumonisin-contaminated grains can result in varied adverse effects in animals, including horses, rabbits, sheep, rats and pigs. *F. moniliforme* in moldy feed, particularly corn , has been associated with livestock deaths for many years. The horse appears to be the species most sensitive to fumonisins, and equine leukoencephalomalacia (ELEM) is the most frequently encountered disease associated with *F. moniliforme* (20-22). ELEM is characterized by liquefactive necrosis of the cerebral hemispheres. Acute porcine pulmonary edema syndrome (PPE) has also been associated with *F.moniliforme* (23).

9. Fumonisins have been associated with liver damage and changes in the levels of certain lipid classes, especially sphingolipids, in all animals studied (24). Kidney lesions were also found in many animals (24-25). Chronic high dietary levels of fumonisins (at least 50 ppm) were associated with liver cancer and decreased life span in female mice and kidney cancer in male Fisher 344 rats without decreased life spans (26). At lower exposures, which were higher than those associated with levels of fumonisins normally detected in U.S. corn crops, no carcinogenic effect was observed. In a smaller study with BD IX male rats exposed to similar fumonisin levels (50ppm), liver cancer resulted (27). Fumonisin was negative in genotoxicity assays (28,29).

10. *Fusarium* culture material, prepared from an isolate of *F. moniliforme (F. vertiicillioides)*, resulted in liver damage or death by heart failure in baboons (30). Long-term studies, in vervet monkeys fed culture material containing fumonisins, resulted in atherogenic effects and liver toxicity (31). Clinical studies with horses and related species, fed rations containing fumonisins, have demonstrated two forms of toxicity: at low exposure levels (8-22ppm in feed) ingested for weeks, fatal brain damage (ELEM) and mild liver damage occurred; and at higher levels (44-200 ppm in feed) ingested for a shorter period of time (days), mild brain damage and severe liver damage were observed (20-22). Fumonisin- containing culture material caused heart failure leading to pulmonary edema when fed to swine (23).

SAMPLING PLANS, ANALYTICAL AND RESIDUE DATA

Sampling plans

11. From statistical studies conducted so far, variances associated with sampling, sample preparation, and analytical steps of a test procedure that measures fumonisin in shelled corn have been estimated (32). Regression equations were developed to predict the variance as a function of fumonisin concentration for each step of the fumonisin test procedure. The variability associated with the test procedure to measure fumonisins in shelled corn is very similar to the variability associated with the test procedure to measure aflatoxins in shelled corn. For small sample sizes, sampling variance is the largest source of the total testing variation, and the variances associated with each step of the test procedure increases with fumonisin

concentration. Further studies are needed to determine the shape of the curve (symmetrical, skewed, etc.) that will best describe the distribution of sample test results from a given lot of corn so that the performance of fumonisin sampling plans can be predicted.

Methods of analysis

12. Many analytical methods have been developed for the identification and quantification of FB_1 and FB_2, the most abundant and toxicologically significant fumonisins. These fumonisins can be separated and analyzed by thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), gas-chromatography-mass spectrometry (GC-MS), capillary electrophoresis and various immunochemical methods. The fumonisins are water-soluble, therefore initial extractions usually involve water with varying ratios of either methanol or acetonitrile. HPLC has been widely used for the analysis of fumonisins. The fumonisins do not have strong UV-absorbing or fluorescing groups, therefore most of the analytical methods require the formation of a stable derivative for detection (33). A reversed-phase HPLC method for the analysis of fluorescence derivatized FB_1 and FB_2 in corn kernels was subjected to an international collaborative study conducted under the auspices of the International Union of Pure and Applied Chemistry (IUPAC) and involved 11 participants from 6 countries (34). This method was subsequently modified, extended to include FB_3, and subjected to a second collaborative study under the auspices of the Commission on Food Chemistry of IUPAC; it has now been accepted as an official AOAC-IUPAC method for corn kernels at concentrations of 500-8000 ng FB_1/g or 800-12800 ng total fumonisins/g[995.15] (35).

13. Methods that have been developed and validated for the extraction and analysis of fumonisins in whole corn kernels cannot effectively be used for processed corn products without further modifications. In conducting surveys involving milled or processed products, it is essential that recovery studies be done for each type of product analyzed in order to determine if changes in fumonisin levels represent real losses of the toxins or represent poor recovery of toxins from the product matrix (36-39). The 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; some can also effectively complement HPLC procedures that are widely used in routine monitoring for fumonisins. Comprehensive reviews of current methodology for fumonisins were recently published (40,41). The lower limit of quantitation of many of the methods currently available is about 0.1 ppm, however, the reported detection limits are usually much lower. No validated analytical methods for fumonisins have been referred to the Codex Committee on Methods of Analysis and Sampling (CCMAS) for consideration and evaluation.

Residue data

14. The worldwide occurrence of fumonisins in corn and corn-based products has been well documented and reviewed in the literature (42-50). The fumonisins are produced mainly in corn and corn-based products, however, the sporadic natural occurrence of fumonisins in sorghum, rice and navy beans has been reported (19,51-54). In reviewing the analytical data in the literature, it should be noticed that some of the data is derived from examining relatively small numbers of samples ; therefore such data may not be adequate for making intake estimates of exposure because of sampling errors associated with the heterogeneous distribution of fumonisins in commodities. Of the many methods that have been developed and used for quantitatively determining fumonisins in foods, only a relative few have been subjected to a formal inter-laboratory (collaborative) study. In some cases, the limit of determination is not given, hence it is difficult to determine the meaning of negative samples or samples labeled “trace”. Even when a well-studied method has been used, recovery data or the description of a procedure to confirm the identity of the analyte is lacking. This is particularly true of some of the data generated by the increasingly popular immunoassay techniques. There is need for more on-going survey data from various countries so that year-to-year variations in fumonisin levels can be noted and possibly correlated with geographic areas and various environmental factors. In spite of the possibility of some errors in the accuracy of the data reported, the available data serves the important function of giving a very general picture of the extent of contamination of the food supply with fumonisins. As advances are made in method development and analytical techniques for fumonisins, investigators will be better able to focus in on more accurate, quantitative data.

15. The levels of toxins found in corn in commercial channels vary from country to country, however, it is generally noted that higher levels are usually found in corn grown in countries with warmer climates (13). The implementation of good agronomic practices can reduce preharvest contamination of corn to some extent, but there are others factors pertaining to the relationship between field infection of corn with *Fusarium* species and the production of fumonisins that are not presently understood. In the U.S., investigators were not able to establish a direct correlation between climatic conditions and fumonisin levels in corn grown in the mid-west over 5 crop years although considerable variability in toxin content was observed (55).

16. Fumonisin levels in raw corn may be reduced as a result of various processing procedures. Lower levels of toxins have been found in milled corn products such as corn meal, and flour, and in products made from these such as corn bread, corn flakes and corn bran cereals. Fumonisins are heat stable, therefore ordinary cooking and heat processes do not substantially reduce their levels in foods. Processed corn products such as corn starch, high fructose corn syrup, corn oil and corn oil margarine do not contain detectable levels of fumonisins. The manufacturing processes involved removes virtually all fumonisins that may be present in the wet-milled fractions from which these products are made (47,56). Ready-to-eat breakfast cereals such as corn flakes and puffed-corn type cereals are manufactured from fractions of dry-milled corn; these products are virtually free of fumonisins. Lower levels of fumonisins have been reported in canned and frozen sweet corn than in unpopped popcorn and field corn (47,57-58). Processing (popping) of unpopped popcorn has been shown to reduce fumonisin levels.

17. Because of their water solubility, fumonisins are less likely to bioaccumulate in animal tissues than lipid-soluble compounds. Fumonisin residues have either not been detected or are detected at extremely low levels in milk, eggs and edible meat (59,60). When detected, fumonisin residues are generally found in organ tissues (i.e., liver and kidney). Low levels of fumonisins have been detected in commercial beer; this is believed to result from the use of corn grits as an adjunct in replacement of , or in addition to the traditional use of barley in the brewing process (61,62). In order to acquire a better database on the baseline levels of fumonisins in corn-based products, Codex Member States should be encouraged to submit available survey data obtained by using validated analytical methods (e.g. AOAC/IUPAC collaboratively studied methods).

LEVELS OF INTAKE

18. Exposure to fumonisins is thought to occur almost exclusively from corn. The amount of intake may vary considerably, with substantial variation in fumonisin levels among corn samples from a particular crop, amount of corn consumed by different individuals, and crop variation from year to year. In areas where corn is a major staple food, daily consumption may exceed 100 g/day, whereas occasional consumption of corn and corn products will typically result in a daily consumption rate of 10 g/day (63). Fumonisin levels may range from less than 1 ppm to over 100 ppm. Corn usually has fumonsin levels below 1 ppm. Episodes of high *Fusarium* proliferation may result in sustained levels of exposure to corn products with average levels in excess of 10 ppm.

19. A decision to impose limits on fumonisins in foods should depend in part on region specific exposure levels. Estimating intakes of fumonisins on a global scale will be difficult because of what is known about the wide variation in some areas (e.g. South Africa, China, and the United States), and the lack of data in many other parts of the world. However, should it become necessary, it should be possible to provide crude estimates of the distribution of global exposure; the range of fumonisin exposure can be gleaned from the information given above. In countries where more extensive food consumption data are available, more realistic exposure estimates may be calculated. In the U.S., for example, most consumers have exposures to fumonisin that are less than 10 μg/day, while consumers with high exposures (90^{th} percentile) may be 21μg/day. Codex Member States should present exposure assessments based on residue data obtained using validated analytical methods for various corn-based products.

Fair Trade Considerations

20. Corn kernels are traded extensively in the international market as raw commodities, which will either be used directly as an ingredient for animal, or undergo further processing. In order to ensure fair trade between those countries that depend heavily on imported corn versus those countries that export corn, Codex should develop science-based standards and guidelines so that an equivalency of burden will exist between the exporter and the importer, e.g, testing variances (including sampling and analysis).

AGRICULTURAL, TECHNOLOGICAL AND COMMERCIAL CONSIDERATIONS

Agricultural Approaches

21. Preharvest management of corn crops is the best way to control and reduce fumonisin levels in corn. The results from limited investigations on agronomic practices indicate that: (a) fungal infection rates are higher in crops planted in fields previously planted with corn, particularly when residues from those crops were left in the field, (b) the incidence of *Fusarium* kernel rot induced by seed borne *F. moniliforme* (resulting in the production of fumonisins) is higher in warm climates under drought conditions, and (c) freshly harvested corn should be dried to a suitable moisture level immediately and stored (8,11,64,65). Fumonisin levels in corn grown in various countries are expected to vary from year to year based on environmental factors as well as the extent of insect invasions. In a study involving the fumonisin levels in corn produced over 5 consecutive crop years in the same geographic area, it was found that there was a consistently high range of FB_1 levels in the crops for the first 4 years (0-37.9 ppm {μg/g}), followed by a low range (0-1.6 ppm) during the 5^{th} year (55,66). The only condition noted during the 5^{th} year that might have contributed to the decreased levels of FB_1 was the occurrence of cool, damp weather during most of the growing season; this might have resulted in less stress on the corn plants and therefore lower levels of toxins being produced.

22. Studies involving the use of commercial hybrids of corn developed by traditional breeding techniques reveal that the hybrids differ in their tendency to accumulate fumonisins due to the influence of environmental factors; furthermore, it has been noted that corn hybrids planted outside their geographic area of adaptation produce higher levels of fumonisin due to the added stress conditions (10,12). Some studies that are underway to reduce fumonisin levels in corn include the development and use of corn genetically engineered for (1), resistance to *Fusarium* infections and (2) for resistance to insects (European Corn Borer) (11,67,68). Early results from these studies indicate that under some conditions genetic engineering of corn can reduce the levels of fumonisins in corn, but further studies are required. *F. moniliforme* is a seed-borne as well as a soil-borne pathogen of corn; therefore, the extent of infection manifested by the fungus in a corn plant depends to some extent on its route of entry (69). An infected corn plant can therefore develop, (a) from a symptomless infection produced by the fungus located in the seed, or (b) as a result of spores from the soil being carried by the wind or insects into the developing corn kernel via the silk tracks. The observation that a significant percent of visually healthy-looking corn kernels may contain fumonisin levels up to 1 ppm or higher, makes it difficult for seed companies to select resistant corn genotypes based on visual symptoms (10,11). The cost of screening large numbers of genotypes for symptomless infections by culturing large numbers of kernels would be extremely expensive. A newer approach to genetic engineering involves the incorporation of genes that encode enzymes that can degrade fumonisins *in planta;* transgenic corn containing one of these genes is currently undergoing field testing (70). Countries, that rely heavily on corn for economic reasons and/or as a staple food in the diet, should encourage the diversion of fumonisin-contaminated corn to non-food uses or to processing and manufacturing facilities that can recover fumonisin-free products.

Decontamination Procedures and the Reduction of Fumonisin Contamination through Processing

23. The fumonisins are believed to be stable during drying and storage, therefore the random, unpredictable occurrence of fumonisins in harvested corn necessitates the exploration of different approaches to minimize the levels to which consumers are exposed. Research efforts currently underway in many laboratories are focused on possible ways of reducing fumonisin levels in harvested corn. Some progress has been made in these endeavors; however, no commercial, large-scale applications have been developed as yet.

Physical Removal

24. Corn screenings (broken corn kernels of varying particle sizes) usually contain about 10 times the fumonisin content of intact corn kernels (55). One study has shown that the removal of corn screenings from bulk shipments of raw, fumonisin-contaminated corn , can result in an overall reduction of 26.2% to 69.4% in the total fumonisin levels of bulk corn (71). In a second study, it was found that cleaning stored corn by sequentially passing the kernels through a cleaning apparatus and then onto a gravity table resulted in a 60% reduction in the total fumonisins originally present (72). Density segregation of naturally contaminated corn using water and different concentrations of sodium chloride in water resulted in the removal of 74% and 86% of the fumonisin content, respectively (73). The presence of fumonisins and *F. moniliforme* in symptomless kernels of corn in commercial channels suggests that novel physical techniques must be developed for separating and removing these kernels because they have a high chance of being incorporated in the food supply.

Milling

25. Wet-milling of corn is a major process used to obtain corn starch for further processing into human food. No fumonisins have been detected in the starch fraction obtained from the wet-milling of fumonisin-contaminated corn; this fraction is further processed for the preparation of high fructose corn syrups and other products (47,56). Other fractions from the wet-milling operations contain fumonisins in the order gluten>fiber>germ (56). Modification of the initial steep water step in this milling procedure, to include the addition of sodium bisulfite, is reported to further reduce fumonisin levels in these fractions, however the degradation products produced have not been characterized (74). Corn oil, for use in human food, is extracted from the germ fraction and refined; no fumonisins have been detected in commercially processed corn oils (47). The germ residue (after oil extraction) along with the gluten and fiber fractions are used as components in animal feed.

26. Dry-milling of corn is a process by which the components of corn kernels are separated into fractions based on particle sizes in roller mills. Generally, the initial steps in the process involves the removal of the pericarp (referred to as the bran fraction) and the germ. The remaining components of the endosperm are separated into the following fractions based on decreasing particle size in the order , flaking grits > regular grits> corn meal > flour (75). Examination of milled fractions obtained from a commercial dry milling operation of fumonisin-contaminated corn, revealed that fumonisin levels were highest in the bran and germ fractions; lowest levels were found in the fractions with larger size particles (e.g. flaking grits) (76).

Heat

27. Fumonisins are heat stable compounds that survive under most conditions used for baking and frying (77). Generally, foods heated to temperatures above 150 ^{0} C. during processing may have reduced levels of fumonisins. Thermally processed corn products (canned corn, tortillas, grits) generally have lower levels of fumonisins than unprocessed corn milled products. Extrusion cooking, under various laboratory conditions, as well as roasting has been observed to reduce fumonisin levels to varying extents (78). Research is needed to identify and characterize the degradation products formed during the thermal processing. Thermal processing might convert the fumonisins to other biologically active forms that may or may not be recoverable from the food matrix or to compounds with undesirable side effects.

Biological Decontamination

28. Ethanol fermentation of fumonisin contaminated corn results in very little degradation of the toxins; most of the toxins remain in the distiller's grains, thin stillage and distiller's soluble fraction. The fermentation process does not destroy fumonisins, so about 85% of the toxins can be recovered in the products. No fumonisin is detected in the distilled alcohol (56,79).

Chemical Decontamination

29. Nixtamalization, is a process used for making masa for tortillas and other corn products. This process involves boiling and soaking corn in a solution of calcium hydroxide; it has been found to increase the niacin availability in corn and also reduce fumonisin levels in contaminated corn. Using this procedure, or variations thereof, the masa obtained has been found to contain not only reduced levels of fumonisins but also other compounds, one of which has been identified as hydrolyzed fumonisin. Toxicological studies of the nixtamalized corn products in laboratory animals have revealed that these products still exert hepatotoxic and nephrotoxic effects (80). Some investigators have reported that the products from the nixtamalized process are as toxic or more toxic than the fumonisins in the untreated corn (81), while others have reported that the hydrolyzed products are less toxic than the untreated corn (80). A modified nixtamalization procedure, incorporating various combinations of hydrogen peroxide and sodium bicarbonate in addition to calcium hydroxide, has been reported to give a 100% reduction of FB_1, however the masa product exhibited about 60% of the toxicity of the untreated corn using a brine shrimp assay procedure (82).These conflicting results may be partially explained based on several factors that have not been adequately studied regarding this processing technique: a) the optimal length of time required for complete nixtamalization to occur has not been established thereby resulting in incomplete removal of the pericarp which would contain higher levels of the fumonisins, (b) various genotypes of corn may have been involved, (c) improved methods are needed for extracting, analyzing and identifying the various compounds formed in nixtamalized corn, and (d) the complex formed by the reaction of calcium with the starch linkage in the corn requires further study (83). To determine the effectiveness of the nixtamalization process, the reaction products produced should be evaluated chemically and toxicologically at each stage in the process.

30. Ammoniation of corn naturally contaminated with fumonisins, or *F. moniliforme* culture material resulted in partial reduction of fumonisin levels , but the ammoniated products remained toxic to animals (84,85).

31. A recent study describes the results of a reaction of the amino group of FB_1 with the reducing sugar, fructose, in a nonenzymatic browning (Maillard) reaction (86). This process resulted in a significant reduction in the level of detectable FB_1 and the reaction product was not toxic to rats and seemed to prevent FB_1-induced hepatotoxicity. Further studies are needed to identify and characterize the FB1-fructose conjugate.

Gamma - Irradiation

32. In a study to investigate the effects of gamma irradiation on naturally contaminated corn flour, 15 kGY effectively sterilized the flour, but caused only about a 20% reduction in its fumonisin content (87).

RISK MANAGEMENT CONSIDERATIONS AND PUBLIC HEALTH CONCERNS

33. Current technology cannot prevent fumonisin contamination of corn crops before harvest. The incidence and levels of fumonisins in corn crops around the world vary considerably depending on many factors including environmental conditions, extent of insect damage, hybrid of corn planted and agronomic practices employed. There is need for much more information on the year-to-year variability of the levels of fumonisins in corn grown in many parts of the world , as well as the consumption patterns of various populations before any long term risk management decisions can be made internationally. The scientific information currently available on the occurrence of fumonisins in corn suggests that immediate research should be focused on the development of control measures embodied in a good agricultural practices (GAPs) program. The implementation of these practices, along with advances in post-harvest techniques involving proper drying and storage conditions followed by good manufacturing practices (GMPs), could substantially reduce the levels of the toxins in the food supply.

34. No official limits have been established for fumonisins in corn. Switzerland has established a level of 1 μg/g in corn products for human consumption (87).

CONCLUSIONS AND RECOMMENDATIONS

35. *Fusarium moniliforme [= F. verticillioides]* is one of the most prevalent seed and soil-borne pathogens associated with corn (*Zea ,mays*) throughout the world. The conditions that favor growth and proliferation of this fungus and hence the production of fumonisins is highly dependent on climatic conditions, therefore it is very difficult to develop an effective procedure to prevent mold growth and fumonisin production. The maintenance of a wholesome food supply is a major responsibility to be shared by the food industry (producers and processors) and the regulatory agencies involved (88). In view of the available toxicological information regarding the various adverse effects noted in animals and humans , it is prudent at this time to develop good agricultural practices (GAPs) and good manufacturing practices (GMPs) to reduce the levels of fumonisins in food.

36. There are no single practical methods available for significantly reducing fumonisin levels in corn at this time. Genetic engineering approaches are the most attractive methods now under development. In order to manage the risk associated with fumonisin contamination in corn, an integrated risk management system approach is needed where preharvest management (including GAP), harvesting management (to include time of harvest, control of temperature and moisture during transportation and storage of corn) and post-harvest management (including GMP, decontamination and diversion strategies) become a routine sequence with appropriate controls incorporated at each level (89). Based on the information presented in this document, it is suggested that CCFAC should consider the following recommendations.

(a) Research on methods to prevent and/or reduce contamination of corn in the field, during storage and processing by *Fusarium* species should be encouraged. There is need for a better understanding of the *Fusarium*-corn interactions in asymptomatic and symptomatic infections in corn in the field.

(b) CCFAC should begin elaborating a code of practice that contains guidance on Good Agricultural Practices (GAP) and Good Manufacturing Practices (GMP) for the reduction of fumonisins in corn.

(c) Codex should develop sampling plans and validated methods of analysis for fumonisins in corn kernels and corn kernel products for endorsement by the Codex Committee on Methods of Analysis and Sampling (CCMAS). The development and validation of additional methods for the quantitative determination of fumonisins in milled and processed corn products should be encouraged.

(d) Codex Member States should be encouraged to submit data from surveys of corn and corn-based products in their country, using validated analytical methodology, over a period of several years to reflect seasonal variations. In order to develop an appropriate and fair international standard, data is needed from all geographical locations; consideration must also be given to regional differences in food consumption patterns when determining exposure estimates.

(e) CCFAC should defer development of international standards until regional data on incidences and levels is available for several years and JECFA performs a risk assessment at its February 2001 meeting.

(f) Research on the development of resistant genotypes of corn should be encouraged by Codex.

(g) Research involving the development of genetically engineered corn that resists *Fusarium* growth or degrade fumonisins *in planta* should be encouraged.

REFERENCES-FUMONISIN

1. Nelson, P.E.; Plattner, R.D.; Shackelford, D.D. and Desjardins, A.E. Production of fumonisins by *Fusarium moniliforme* strains from various substrates and geographic areas. Appl. Environ. Microbiol. 57(8): 2410-2412, 1991.
2. Bacon, C.W.; Bennett, R.M.; Hinton, D.M. and Voss, K.A. Scanning electron microscopy of *Fusarium moniliforme* within asymptomatic corn kernels and kernels associated with equine leukoencephalomalacia. Plant Disease 76(2): 144-148, 1992.
3. Sydenham, E.W.; Shephard, G.S.; Thiel, P.G.; Marasas, W.F.O.; Stockenstrom, S. Fumonisin contamination of commercial corn-based human foodstuffs. J. Agric. Food Chem. 39: 2014-2018, 1991.
4. Bezuidenhout, S.C.; Gelderblom, W.C.A. ; Gorst-Allman, C.P.; Horak, R.M.; Marasas, W.F.O.; Spiteller, G.; Vleggaar, R. Structure elucidation of the fumonisins, mycotoxin from *Fusarium moniliforme*.. Chem. Soc. Chem. Commun. 743-745, 1988.
5. Musser, S.M and Plattner, R.D. Fumonisin composition in cultures of *Fusarium moniliforme, Fusarium proliferatum,* and *Fusarium nygami* . J. Agric Food Chem. 45: 1169-1173, 1997.
6. Sydenham, E.W.; Shephard, G.S.; and Thiel, P.G. Liquid chromatography determination of fumonisins B_1, B_2 and B_3 in foods and feeds. J Assoc. Off. Anal. Chem. 75: 313-318, 1992.
7. Thiel, P.G., Marasas, W.F.O.; Sydenham, E.W., Shephard, G.S. and Gelderblom, W.C.A. The implications of naturally occurring levels of fumonisins in corn for human and animal health. Mycopathologia 117: 3-9, 1992.
8. Bacon, C.W. and Nelson, P.E.; Fumonisin production in corn by toxigenic strains of *Fusarium moniliforme* and *Fusarium proliferatum.* J. Food Prot. 57(6): 514-521, 1994.
9. Munkvold, G.P.; McGee, D.C. and Carlton, W.M. Importance of different pathways for maize kernel infection by *Fusarium moniliforme.* Phytopathology 87(2): 209-217, 1997.
10. Doko, M.B.; Rapior, S; Visconti, A and Schjth, J.E. Incidence and levels of fumonisin contamination in maize genotypes grown in Europe and Africa. J. Agric. Food Chem. 43: 429-434, 1995.
11. Munkvold, G.P. and Desjardins, A.E. Fumonisins in maize - Can we reduce their occurrence? Plant Disease 81(6):556-565 ,1997.
12. Shelby, R.A.; White, D.G.; Bauske, E.M. Differential fumonisin production in maize hybrids. Plant Disease 78: 582-584, 1994.
13. Miller, J.D. Factors affecting the occurrence of fumonisin in corn. Abstracts of Papers (p.21)-International Conference on the Toxicology of Fumonisin. June 28-30, 1999. Arlington, VA.
14. Ross, P.F.; Rice, L.G.; Osweiler, G.D.; Nelson, P.E.; Richard, J.L.; Wilson, T.M. A review and up-date of animal toxicoses associated with fumonisin-contaminated feeds and production of fumonisins by *Fusarium* isolates. Mycopathologia 117: 109-114, 1992.
15. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. *Some Naturally Occurring Substances: Food Items and Constituents, Heterocyclic Aromatic Amines and Mycotoxins* (1993) Vol.56: 445-466 IARC/WHO.
16. Rheeder, J.P.; Marasas, W.F.O.; Thiel, P.G.; Sydenham, E.W.; Shephard, G.S.; and Van Schalkwyk, D.J. Fusarium moniliforme and fumonisins in corn in relation to human esophageal cancer in Transkei. Phytopathology 82: 353-357, 1992.
17. Chu, F.S. and Li, G.Y. Simultaneous occurrence of fumonisin B_1 and other mycotoxins in moldy corn collected from the People's Republic of China in regions with high incidence of esophageal cancer. Appl. Environ. Microbiol. 60: 847-852, 1994.
18. Franceschi, S.; Bidoli, E.; Buron, A.E.; and La Vecchia, C. Maize and risk of cancer in the oral cavity, pharynx and esophagus in northeastern Italy. J Natl. Cancer Instit. 82: 1407-1411, 1990.
19. Bhat, R.V.; Shetty, P.H.; Amruth, R.P. and Sudershan, R.V. A foodborne disease outbreak due to the consumption of moldy sorghum and maize containing fumonisin mycotoxins. Clin. Toxicol 35(3): 249-255, 1997.
20. Kellerman, T.S.; Marasas, W.F.O.; Thiel, P.G.; Gelderblom, W.C.A.; Cawood, M. and Coetzer, J.A.W. Leukoencephalomalacia in two horses induced by oral dosing of fumonisin B_1. Onderstepoort J. Vet. Res. 57: 269-275, 1990.
21. Wilson, T.M.; Ross, P.E.; Owens, D.L.; Rice, L.G.; Green, S.A.; Jenkins, S.J.; and Nelson, H.A. Experimental reproduction of ELEM – a study to determine the minimum toxic dose in ponies. Mycopathologia 117: 115-120, 1992.

22. Ross, P.F.; Ledet, A.E.; Owens, D.L.; Rice, L.G.; Nelson, H.A.; Osweiler, G.D.; and Wilson, T.M. Experimental equine leukoencephlomalacia, toxic hepatosis and encephalopathy caused by corn naturally contaminated with fumonisins. J. Vet. Diagn. Invest. 5: 69-74, 1993.
23. Smith, G.W.; Constable, P.D.; Tumbleson, M.E.; Rottinghaus, G.E. and Haschek, W.M. Sequence of cardiovascular changes leading to pulmonary edema in swine fed fumonisin-containing culture material. Am J Vet. Res. 60(10): 1292-1300, 1999.
24. Merrill, A.H.; Schmelz, E.M.; Dillehay, D.L.; Spiegel, S.; Shayman, J.A.; Schroeder, J.J.; Riley, R.T.; Voss, K.A. and Want, E. Sphingolipids—the enigmatic lipid class: biochemistry, physiology, and pathophysiology. Toxicol Appl. Pharmacol. 142: 208-225, 1997.
25. Norred, W.P. Voss, K.A.; Riley, R.T.; Meredith, F.I.; Bacon, C.W. and Merrill, A.H.,Jr. Mycotoxins and health hazards: toxicological aspects and mechanism of action of fumonisins. J. toxicol. Sci. 23(Suppl. II): 160-164, 1998.
26. NTP (National Toxicology Program). NTP Technical Report on the Toxicology and Carcinogenesis Studies of fumonisin B_1 in F344/N rats and B6C3F_1 mice (Feed Studies). NTP TR 496. NIH Publication No. 99-3955. U.S. Department of Health and Human Services, NIEHS, P.O. Box 12233, MD E1-02, Research Triangle, NC 27709, May 1999.
27. Gelderblom, W.C.A.; Kriek, N.P.J.; Marasas, W.F.O.; Thiel, P.G. Toxicity and carcinogenicity of the *Fusarium moniliforme* metabolite, fumonisin B_1, in rats. Carcinogenesis 12: 1247-1251, 1991.
28. Gelderblom, W.C.A.; Semple, E.; Marasas, W.F.O.; and Farber, E. The cancer-initiating potential of the fumonisin B mycotoxins. Carcinogenesis 13(3): 433-437,1992.
29. Norred, W.P.; Plattner, R.D.; Vesonder, R.F.; Bacon, C.W. and Voxx, K.A. Effects of selected secondary metabolites of *Fusarium moniliforme* on unscheduled synthesis of DNA by rat primary hepatocytes. Food Chem. Toxicol. 30: 233-237, 1992.
30. Kriek, N.P.J.; Kellerman, T.S.; and Marasas, W.F.O. A comparative study of the toxicity of *Fusarium verticillioides* (=*F.moniliforme*) to horses, primates, pigs, sheep and rats. Ondersterpoort J Vet. Res. 48: 129-131, 1981.
31. Fincham, J.E.; Marasas, W.F.O.; Taljaard, J.J.F.; Kriek, N.P.J.; Badenhorst, C.J.; Gelderblom, W.C.A.; Seier, J.V.; Smuts, C.M.; Faber, M.; Weight, M.J.; Slazus, W.; Woodroof, C.W.; Van Wyk, M.J.; Kruger, M. and Thiel, P.G. Atherogenic effects in a non-human primate of *Fusarium moniliforme* cultures added to a carbohydrate diet. Atherosclerosis 94: 13-25, 1992.
32. Whitaker, T.B.; Trucksess, M.W.; Johansson, A.S.; Giesbrecht, F.G.; Hagler, W.M.; and Bowman, D.T. Variability associated with testing shelled corn for fumonisin. J AOAC Intl. 81(6): 1162-1168, 1998.
33. Pohland, A.E. Worldwide occurrence of fumonisins. IN: *The Toxicology Forum*; Caset Assoc. Ltd.: Fairfax, VA 1994 pp 186-196.
34. Thiel, P.G.; Sydenham, E.W.; Shephard, G.S. and van Schalwwyk, D.J. Study of reproducibility characteristics of a liquid chromatographic method for the determination of fumonisins B_1 and B_2 in corn: IUPAC collaborative study. J. AOAC Intl 76(2): 361-366, 1993.
35. Sydenham, E.W.; Shephard, G.S.; Thiel, P.G.; Stockenstroem S.; Snijman, P.W. Liquid chromatographic determination of fumonisins B_1, B_2 and B_3 in corn: AOAC/IUPAC collaborative study. J. AOAC Int. 79:688-696, 1996.
36. Scott, P.M. Fumonisins Intl. J. Food Microbiol. 18: 257-270, 1993.
37. Bullerman, L.B. and Tsai, W-Y. J.; Incidence and levels of *Fusarium moniliforme, Fusarium proliferatum* and fumonisins in corn and corn-based foods and feeds. J. Food Prot. 57(6):541-546, 1994.
38. Scott, P.M. and Lawrence, G.A. Stability and problems in recovery of fumonisins added to corn-based foods. J. AOAC Intl. 77(2): 541-545, 1994.
39. Castelo, M.M. Extrusion cooking reduces recoverability of fumonisin B_1 from extruded corn grits. J. Food Sci 63(4): 696-698, 1998.
40. Trucksess, M.W. and Abouzied, M.M. Evaluation and application of immunochemical methods for fumonisin B_1 in corn. IN:*Immonoassays for Residue Analysis. Food Safety*. R.C. Beier and L.H. Stanker, EDS. ACS Symposium Series 621, American Chemical Society, Washington, D.C. pp358-367, 1996.
41. Wilson, D.M.; Sydenham, E.W.; Lombaert, G.A.; Trucksess, M.W.; Abramson, D. and Bennett, G.A. Mycotoxin Analytical Techniques. IN: *Mycotoxins in Agriculture and Food Safety*, K.K.Sinha and D. Bhatnager EDS. Marcel Dekker, Inc. New York, N.Y. pp135-182, 1998.
42. Doko, M.B.; Visconti, A. Occurrence of fumonisins B_1 and B_2 in corn and corn-based human foodstuffs in Italy. Food Addit. Contam. 11: 433-439, 1994.

43. Marasas, W.F.O. Fumonisins: history, world-wide occurrence and impact. IN: *Fumonisins in Food*. L.S. Jackson, J.W. DeVries, and L.B. Bullerman, eds. Plenum Press, New York , pp 1-18, 1996.
44. Bullerman, L.B. Occurrence of *Fusarium* and fumonisins on food grains and in foods. IN: *Fumonisins in Foods.* L.S.Jackson, J.W.DeVries, and L.B.Bullerman, eds. Plenum Press, New York, pp 27-38, 1996.
45. Pohland, A.E. Occurrence of fumonisins in the U.S. food supply. IN: *Fumonisins in Foods.* L.S.Jackson, J.W. DeVries, and L.B. Bullerman, eds. Plenum Press, New York, pp 19-26, 1996.
46. Shephard, G.S.; Thiel, P.G.; Stockenstroem, S.; and Sydenham, E.W. Worldwide survey of fumonisin contamination of corn and corn-based products . J. AOAC Int. 79:671-687, 1996.
47. Patel, S.; Hazel, C.M.; Winterton, A.G.M., and Gleadle, A.E. . Surveillance of fumonisins in UK maize-based foods and other cereals. Food Addit. Contam. 14(2): 187-191, 1997.
48. Castelo, M.M.; Sumner, S.S., and Bullerman, L.B. Occurrence of fumonisins in corn-based food products. J. Food Prot. 61(6): 704-707, 1998.
49. De Nijs, M; Sizoo, E.A.; Vermunt, A.E.M.; Notermans, S.H.W.; and van Egmond, H.P. The occurrence of fumonisin B_1 in maize-containing foods in the Netherlands. Food Addit. Contam. 15(4): 385-388,1998.
50. Solovey, M.M.S.; Somoza, C.; Cano, G.; Pacin, A.and Resnik, S. A survey of fumonisins, deoxynivalenol, zearalenone and aflatoxins contamination in corn-based food products in Argentina. Food Addit. Contam. 16(8): 325-329,1999.
51. Tseng, T.C., Tu, J.C. and Soo, L.C. Natural occurrence of mycotoxins in Fusarium infected beans. Microbios. 84:21-28,1995.
52. Patel, S.; Hazel, C.M.; Winterton, A.G.M.; and Mortby, E. Survey of ethnic foods for mycotoxins. Food Addit. Contam. 13:833- 841, 1996.
53. Munibazi C. and Bullerman, L.B. Molds and mycotoxins in foods in Burundi. J Food Prot. 59: 869-875,1996.
54. Abbas, H.K., Cartwright, R.D., Shier, W.T., Abouzied, M.M., Bird, C.B.,Rice, L.G., Ross, P.F., Sciumbato, G.L., and Meredith, F.I. Natural occurrence of fumonisins in rice and *Fusarium* sheath rot disease. Plant Disease 82(1): 22-25, 1998.
55. Murphy, P.A.; Rice, L.G.; Ross, P.F. Fumonisins B_1, B_2, and B_3 content of Iowa, Wisconsin, and Illinois corn and corn screenings. J. Agric. Food Chem. 41: 263-266, 1993.
56. Bennett, G.A. and Richard, J.L. Influence of processing on Fusarium mycotoxins in contaminated grains. Food Technology 50(5), 235-238, 1996.
57. Trucksess, M.W., Stack, M.E., Allen, S. and Barrion, N. Immunoaffinity column coupled with liquid chromatography for determination of fumonisin B_1 in canned and frozen sweet corn. J.AOAC Intl. 78(3): 705-710, 1995.
58. Food and Drug Administration (FDA), Unpublished findings, 1999.
59. Prelusky, D.B.; Trenholm, H.L.; Rotter, B.A.; Miller, J.D.; Savard, M.E.; Yeung, J.M.; and Scott, P.M. Biological fate of fumonisin B_1 in food-producing animals. IN: *Fumonisins in Food*. L.S. Jackson, J.W. DeVries, and L.B. Bullerman, eds. Plenum Press, New York, pp 265-278, 1996.
60. Miller, M.A.; Honstead, J.P., and Lovell, R.A. Regulatory aspects of fumonisins with respect to animal feed. IN: *Fumonisins in Food.* L.S. Jackson; J.W. DeVries and L.B. Bullerman eds. Plenum Press, New York, pp 363-368, 1996.
61. Scott, P.M. and Lawrence, G.A. Analysis of beer for fumonisins. J. Food Prot. 58(12): 1379-1382,1995.
62. Hlywka, J.J. and Bullerman, L.B. Occurrence of fumonisin B_1 and B_2 in beer. Food Addit. Contam 16(8): 319-324,1999.
63. World Health Organization (WHO), Food Safety Unit, Programme of Food Safety and Food Aid. GEMS/FOOD Regional Diets. Geneva: WHO, 1998.
64. Warfield, C.Y. and Gilchrist, D.G. Influence of kernel age on fumonisin B_1 production in maize by *Fusarium moniliforme*. Appl. Environ. Microbiol. 65(7): 2853-2856, 1999.
65. Miller, J.D. Epidemiology of *Fusarium* ear diseases of cereals. IN: *Mycotoxins in Grain-Compounds Other Than Aflatoxin*. J.D. Miller and H.L.Trenholm eds. Eagan Press, St. Paul, MN, pp 19-36, 1994.
66. Rice, L.G. and Ross, P.F. Methods for detection and quantitation of fumonisins in corn, cereal products and animal excreta. J. Food Prot. 57(6): 536-540, 1994 .
67. Munkvold, G.P.; Hellmich, R.L. and Rice, L.G. Comparison of fumonisin concentrations in kernels of transgenic Bt maize hybrids and nontransgenic hybrids. Plant Disease 83(2), 130-138, 1999.

68. Munkvold, G.P.; Hellmich, R.L. and Showers, W.B. Reduced *Fusarium* ear rot and symptomless infection in kernels of maize genetically engineered for european corn borer resistance. Phytopathology 87(10): 1071-1077, 1997.
69. Riley R.T., Norred, W.P. and Bacon C.W. Fungal toxins in foods: recent concerns. Annu. Rev. Nutr. 13:167-189, 1993.
70. Duvick J. Prospects for reducing fumonisin contamination through genetic modification of maize. Abstracts of Papers (p.24) –International Conference of the Toxicology of Fumonisin, June 28-30, 1999, Arlington, VA.
71. Sydenham, E.W.; Van der Westhuizen, L.; Stockenstrom, S.; Shephard, G.S. and Thiel, P.G. Fumonisin-contaminated maize: physical treatment for the partial decontamination of bulk shipments. Food Addit Contam. 11(1): 25-32,1994.
72. Malone, B.M.; Richard, J.L.; Romer, T.; Johansson, A.S. and Whitaker, T. Fumonisin reduction in corn by cleaning during storage discharge. IN: *Proceedings of the 48th Australian Cereal Chemistry Conference.* L. O'Brian, A.B. Blakeney, A.S. Ross and C.W. Wrigley eds. Cairns, Australia, pp372-379, August 1998.
73. Shetty, P.H. and Bhat, R.V. A physical method for segregation of fumonisin-contaminated maize. Food Chem. 66:371-374,1999.
74. Pujol, R.; Torres, M.; Sanchis, V. and Canela, R. Fate of fumonisin B_1 in corn kernel steeping water containing SO_2. J. Agric. Food Chem. 47:276-278, 1999.
75. Alexander, R.J. Corn dry milling: processes, products and applications. IN: *Corn: Chemistry and Technology.* S.A. Watson and P.E. Ramstad, eds. Am. Assoc. Cereal Chem.: St. Paul, MN.pp 351-376, 1987.
76. Katta, S.K.; Cagampang, A.E.; Jackson, L.S. and Bullerman, L.B. Distribution of *Fusarium* molds and fumonisins in dry-milled corn fractions. Cereal Chem. 74(6): 858-863, 1997.
77. Jackson, L.S.; Katta, S.K.; Fingerhut, D.D.; DeVries, J.W.; and Bullerman, L.B. Effects of baking and frying on the fumonisin B1 content of corn-based foods. J. Agric Food Chem. 45:4800-4805, 1997.
78. Katta, S.K., Jackson, L.S., Sumner, S.S., Hanna, M.A. and Bullerman, L.B. Effect of temperature and screw speed on staability of fumonisin B_1 in extrusion-cooked corn grits. Cereal Chem. 76(1): 16-20,1999.
79. Bothast, R.J.; Bennett, G.A.; Vancauwenberge, J.E. and Richard, J.L. Fate of fumonisin B_1 in naturally contaminated corn during ethanol fermentation. Appl. Environ. Microbiol. 58:233-236,1992.
80. Voss, K.A.; Bacon, C.W.; Meridith, F.I. and Norred, W.P. Comparative subchronic toxicity studies of nixtamalized and water-extracted Fusarium moniliforme culture material. Food Chem. Toxicol 34:623-632, 1996.
81. Hendrich, S.; Miller, K.A.; Wilson, T.M. and Murphy, P.A. Toxicity of *Fusarium proliferatum*-fermented nixtamalized corn-based diets fed to rats: effect of nutritional status. J.Agric Food Chem. 41: 1649-1654, 1993.
82. Park, D.L.; Lopez-Garcia, R.; Truuujillo-Preciado, S.; and Price, R.L. Reduction of risks associated with fumonisin contamination in corn. IN: *Fumonisins in Food.* L.S.Jackson, J.W. DeVries and L.B. Bullerman eds. Plenum Press, New York, pp. 335-344, 1996.
83. Dombrink-Kurtzman, M.A. and Dvorak, T.J. Fumonisin content in masa and tortillas from Mexico. J. Agric. Food Chem. 47: 622-627, 1999.
84. Norred, W.P.; Voss, K.A.; Bacon, C.W. and Riley, R.T. Effectiveness of ammonia treatment in detoxification of fumonisin-contaminated corn. Fd. Chem. Toxicol. 29(12): 815-819, 1991.
85. Park, D.L.; Rua ,S.M.; Mirocha, C.J.; Adb-Alla, E.-S.A.M.; and Weng, C.Y. Mutagenic potentials of fumonisin contaminated corn following ammonia decontamination procedure. Mycopathologia 117:105-108, 1992.
86. Lu, Z.; Dantzer, W.R.; Hopmans, E.C.; Prisk, V.; Cunnick, J.E.; Murphy, P.A.; and Hendrich, S. Reaction with fructose detoxifies fumonisin B_1, while stimulating liver-associated natural killer cell activity in rats. J. Agric. Food Chem. 45: 803-809, 1997.
87. Visconti, A., Solfrizzo, M.; Doko, M.B.; Boenke, A. ; and Pascale,M. Stability of fumonisins at different storage periods and temperatures in gamma-irradiated maize. Fd. Addit. Contam. 13(8): 929-938, 1996.
88. Wood, G.E. and Trucksess, M.W. Regulatory control programs for mycotoxin-contaminated food. IN: *Mycotoxins in Agriculture and Food Safety*. K.K.Sinha and D. Bhatnagar eds. Marcel Dekker, Inc. New York pp.459-481, 1998.

89. Lopez-Garcia R. and Park, D.L. Effectiveness of postharvest procedures in management of mycotoxin hazards. IN: *Mycotoxins in Agriculture and Food Safety*. K.K.Sinha nd D. Bhatnagar eds. Marcel Dekker, Inc. New York, pp. 407-433, 1998.