CODEX ALIMENTARIUS COMMISSION

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DISCUSSION PAPER ON FUMONISINS IN MAIZE AND MAIZE-PRODUCTS: CODE OF PRACTICE AND ANY OTHER MEASURES TO CONTROL FUMONISINS IN MAIZE

In order to assist the Committee on how to proceed further with fumonisin contamination in maize and maize products (code of practice and other measures to control fumonisins in these products), Codex members and Observers are invited to consider the conclusions and recommendations on pages 4-5.

BACKGROUND

1. The 6th Session of the Committee on Contaminants in Foods (CCCF) discussed the paper Proposed Draft Maximum Levels for Fumonisins in Maize and Maize-Products and Associated Sampling Plans.

2. Although several delegations expressed their support for the proposed MLs of 5000 µg/kg for unprocessed corn/maize, and 2000 µg/kg for corn/maize flour/meal, the Delegation of Tanzania, supported by many other African delegations indicated that maize was a staple food in their countries and that consumption could be as high as 500 g/person/day and that the PMTDI of fumonisins of 2 µg/kg/bw/day would be exceeded when maize containing 2000 µg/kg or more was consumed. These delegations did not support the proposed MLs and expressed the view that if it was not possible to establish levels that could provide equal protection globally, then establishment of levels should be left to each country to develop based on their consumption patterns. Some of these delegations proposed as an alternative, the development of a code of practice specifically for fumonisins in maize.

3. In view of the discussion, the Committee agreed:

- To develop a discussion paper to identify the gaps in the Code of Practice for Prevention and Reduction of Mycotoxin Contamination in Cereals and the need for a separate code of practice for fumonisins in maize and whether there are any other measures to control fumonisins in maize; and

- To suspend work on MLs for fumonisins in maize and its associated sampling plans for 1 year until the outputs of the discussion paper are considered.

4. The Committee agreed to establish an electronic Working Group lead by Brazil and co-chaired by the United States of America to develop the discussion paper. African countries were encouraged to participate in this working group.[1]

5. Brazil and the United States of America prepared the draft, with comments from Austria, Canada, Colombia, Costa Rica, Sudan and ICGMA. A list of countries and NGOs that joined the EWG can be found in Appendix

PREVENTION AND REDUCTION OF MYCOTOXIN CONTAMINATION IN CEREALS

6. The Code of Practice for the Prevention and Reduction of Mycotoxin Contamination in Cereals (CAC/RCP 51-2003) was established in an attempt to control and manage mycotoxin contamination worldwide. The Code states the importance of implementing good agricultural practices (GAP) and good manufacturing practices (GMP) by producers, and indicated the adoption of a complementary management system to be considered in the future, the Hazard Analysis Critical Control Point (HACCP) system.

[1] REP12/CF, paras. 92-96.

7. The Code contains general principles for the reduction of mycotoxins in cereals during planting, pre-harvest, harvest, storage and transport from storage. In Annex 2 of the Code, there are specific provisions for prevention and reduction of contamination by fumonisins in cereal grains. Some of these recommendations are shown below.

8. **Planting:** Consider developing and maintaining a crop rotation schedule to avoid planting the same commodity in a field in two consecutive years. Wheat and maize have been found to be particularly susceptible to *Fusarium* species and they should not be used in rotation with each other. Crops such as potato, other vegetables, clover and alfalfa that are not hosts to *Fusarium* species should be used in rotation to reduce the inoculum in the field.

9. **Pre-harvest:** Excess precipitation during anthesis (flowering) makes conditions favourable for dissemination and infection by *Fusarium* spp.; thus irrigation during anthesis and during the ripening of the crops, specifically wheat, barley, and rye, should be avoided. Plan to harvest grain at low moisture content and full maturity, unless allowing the crop to continue to full maturity would subject it to extreme heat, rainfall or drought conditions. Delayed harvest of grain already infected by *Fusarium* species may cause a significant increase in the mycotoxin content of the crop.

10. **Harvest:** The time of harvest for maize should be carefully planned. It has been shown that maize grown and harvested during warm months may have fumonisin levels significantly higher than maize grown and harvested during cooler months of the year. Cereals should be dried in such a manner that damage to the grain is minimized and moisture levels are lower than those required to support mold growth during storage (generally less than 15%).

11. Freshly harvested cereals should be cleaned to remove damaged kernels and other foreign matter. Kernels containing symptomless infections cannot be removed by standard cleaning methods. Seed cleaning procedures, such as gravity tables, may remove some infected kernels. More research is needed to develop practical procedures for separating symptomless infected kernels from those that are not infected. This is necessary to prevent further growth of a number of fungal species that may be present on fresh grains, especially *Fusarium* species.

Additional approaches to prevent fumonisin contamination in maize grain

12. Some studies have been conducted worldwide to investigate additional actions and approaches that could help minimize *Fusarium* infestation and fumonisins contamination in maize. Some of these studies are summarized in this discussion paper. Measures include biological control and predictive models.

Biological control

13. Nayaka et al. (2010) showed that maize seed treated with suspension of *Trichoderma harzianum* followed by spray treatment with pure *T. harzianum* culture suspension reduced the levels of fumonisins in all maize cultivars by 56.4-85.8%.

14. Heinl et al. (2010) investigated the effect of recombinant enzymes on the degradation of fumonisin B1 (FB1). The recombinant carboxylesterase was shown to catalyze the deesterification of FB1 to hydrolyzed FB1 and the heterologously expressed aminotransferase was shown to deaminate hydrolyzed FB1 in the presence of pyruvate and pyridoxal phosphate. The results of the work provide a basis for the development of an enzymatic detoxification process for fumonisin B1 in food and animal feed.

15. Pereira et al. (2011), isolated *Bacillus amyloloquefaciens* and *Microbacterium oleovorans* from maize and tested their potential to reduce FB1 levels in maize kernels co-inoculated with *Fusarium verticillioides*. FB1 reduction ranged up to 94.4% in grains treated with *B. amyloloquefaciens,* and up to 81.5% in grains treated with *M. oleovorans*.

16. The effect of *Pediococcus pentosaceus* (strain LOO6) on fumonisins biosynthesis by *F. verticillioides* was investigated by Dalié et al. (2012). *P. pentosaceus* produced some extracellular metabolites (MRS medium) capable of a significant reduction of fumonisin production (75-80.0% after 20 days of incubation), both in liquid medium as in maize kernels. However, under certain conditions, the bacterial strain used could also enhance fumonisins production.

Predictive models

17. De La Campa et al. (2005) constructed a preliminary empirical model to predict fumonisin concentration in maize at harvest based on regression analyses of field data collected in Argentina and the Philippines. The variability of fumonisins was explained mainly by location or weather (47%) and insect damage to the ears (17%). Overall, more than 82% of the variability of fumonisin content in maize was explained by the model.

18. Battilani et al. (2008) evaluated the role of the cropping system on fumonisin levels in northern Italy to contribute to the development of a predictive system for fumonisin contamination. In the 6 year period from 2002 to 2007, 438 maize samples were collected in five regions, supported by agronomic data, and analyzed for fumonisin content. The logistic regression model developed explained 60% of the variability of fumonisins levels in maize, with major roles for longitude, maturity class and growing weeks contributing the most. This model did not include meteorological information.

19. Maiorano et al (2009) presented a preliminary version of FUMAgrain, a dynamic risk assessment model developed with data from the northern regions of Italy. The elements of the pathosystem are simulated by three submodels: (i) maize development, (ii) *F. verticillioides* infection and fumonisins synthesis, (iii) European Corn Borer wounding activity on maize grain. Inputs to the model are (i) planting date, (ii) hourly meteorological data including temperature, relative humidity, wind speed and rain intensity, (iii) information on the phenological development of the hybrid planted (flowering and dry-down), and (iv) information about the chemical treatment against European Corn Borer. FUMAgrain gives an initial risk alert at the end of flowering based on the meteorological conditions during this phase. A second alert follows maturation when an assessment is made from (i) maize grain moisture, (ii) European Corn Borer damage to the ear, and (iii) fumonisin synthesis risk. FUMAgrain demonstrated good capability to simulate fumonisin synthesis in maize grain in Italy and its usefulness for determining the optimal harvest date while respecting grain safety levels required by the international market.

20. Froment et al (2011) described Qualimetre®, a mycotoxin prediction model based on different agro-climatic statistical models using data of maize (DON, zearalenone and fumonisins) and wheat (DON) production in France and Belgium. This tool was proposed to be used on line by grain purchasers and provides a probability of acceptability for each plot at a mycotoxin threshold

21. Torelli et al (2012) developed an artificial neural network (ANN) model suitable for predicting fumonisins, deoxynivalenol and zearalenone contamination of maize at harvest time. Irrigation, chemical treatment against the European corn borer and harvest date significantly affected the level of fumonisin contamination ($P < 0.05$). The authors concluded that the model has the potential for the development of a new approach for the rapid cataloging of grain plot according to fumonisin levels.

FATE OF FUMONISINs DURING PROCESSING

22. In the Annex 2 of the Code of Practice (Prevention and reduction of contamination by fumonisins in cereal grains) it is stated that GAP includes methods to reduce *Fusarium* infection and fumonisin contamination of cereals during planting, harvest, storage, transport and processing. The Code does not elaborate further on processing aspects, which will be also covered in this discussion paper.

23. The fate of fumonisins during processing is affected by many factors, including temperature, moisture of the product, the toxin concentration in the raw product and the presence of other ingredients in the processed food. Processing operations include sorting, milling (dry and wet), heat, extrusion and nixtamalization. In this paper, the discussion will focus on the first two processing operations.

Sorting and cleaning

24. Sorting and cleaning may lower fumonisin levels by removing the contaminated material. Broken maize kernels contain nearly 10 times higher levels of fumonisins than intact ones. Strategies to separate healthy from contaminated kernels include removing the contaminated maize in the buoyant fraction after treatment with saturated sodium chloride solution (Shetty & Bhat, 1999), and sequentially passing stored maize kernels through cleaning equipment followed by a gravity table (Malone et al., 1998).

25. Afolabi et al. (2006) collected maize samples from different farms in Nigeria that had been sorted by farmers as either good quality or poor quality. Twelve of 13 poor quality samples contained fumonisins (1.4 to 110 µg/g), as did the five good quality samples (0.2 to 3.7 µg/g). The authors suggested that the visible sorting of grain as a technique to reduce the exposure of subsistence farmers to fumonisins could be successful if there were enough good quality grain available to permit the poor quality grain to be used for another purpose or discarded.

26. Van der Westhuizen et al (2011) conducted a study to reduce fumonisins levels in home-grown maize based on customary methods used by some South African rural populations to sort visibly infected maize kernels from good maize kernels and to wash the good kernels prior to cooking. The laboratory optimized sorting reduced fumonisin levels by 71% and an additional 13% reduction was achieved with the 10 min ambient temperature water wash.

27. Firrao et al. (2010) presented a new approach for early identification of maize contaminated with fumonisins, based on digital images. Maize samples were imaged under 10 different LED lights (emissions ranging from 720 to 940 nm) and it was possible to establish a correlation between the image derived data with the level of fumonisins in the sample (sum of FB1 and FB2). The authors stated that the method developed produce reliable fumonisins contamination estimation, within a few minutes, requiring minimal equipment, and may be used to assist in selecting lots during maize processing.

Milling (wet and dry)

28. Fumonisins are distributed in milling streams approximately according to their occurrence in the maize seed structure. Wet-milling is used to obtain maize starch, germ and fibres. Commercially processed corn oil obtained from germ fraction does not contain fumonisins (Patel et al. 1997).

29. Dry-milling gives rise to the bran (obtained from the removal of pericarp) and the germ fractions, followed by the fractions obtained by decreasing particle size - grits, corn meal and flour. Fumonisins are not expected to be destroyed during this process and are found in all fractions, with higher concentration in bran and germ (Katta et al. 1997; Brera et al, 2004; Vanara et al., 2009).

30. Resnik (2006) showed that germ and bran had fumonisins levels 29 fold higher than corn meal and corn grits, 13 fold higher than corn flour, and 3 fold higher than whole maize. Hence, in addition to the whole kernel fumonisin content, the contamination of food products depends whether the fractions were obtained from degermed corn (lower levels) or non-degermed or partially-degermed corn (higher levels) (Vanara et al., 2009).

31. Scudamore & Patel (2009) found that grits and flours, which are mostly derived from the endosperm, contain the lowest mycotoxin levels, which are closely related to particle size. Levels of fumonisins in the milled products vary greatly with the milling conditions and the nature and condition of each maize consignment. The levels found in maize flour could represent from 26 to 310% of that present in the initial maize grain.

32. Pietri et al. (2009) evaluated the distribution of fumonisins in fractions derived from dry-milling of contaminated maize. The cleaning step reduced FB1 levels by 11 to 34% and subsequent removal of bran and germ led to a further decrease in contamination levels in the products destined for human consumption. FB1 was concentrated in the finer size fractions and in the inner layers of the kernels.

33. The Food Safety Objective (FSO) concept is the maximum frequency and/or concentration of the hazard in a food at the time of consumption (ICMSF, 2002; CAC, 2007). The FSO framework for fumonisin in maize (Figure 1; Pitt et al. 2013) is a risk management tool for explaining the ecology of toxin formation and the control measures available to manage its levels in foods. If fumonisin is present in maize, the main steps for meeting the FSO in the process includes visual inspection of lots for fungal damage, fumonisin analyses, rejection of lots not meeting the specifications, milling and nixtamalization. If rejection of lots is not applicable, sorting and cleaning are also effective to reduce fumonisin in maize grains.

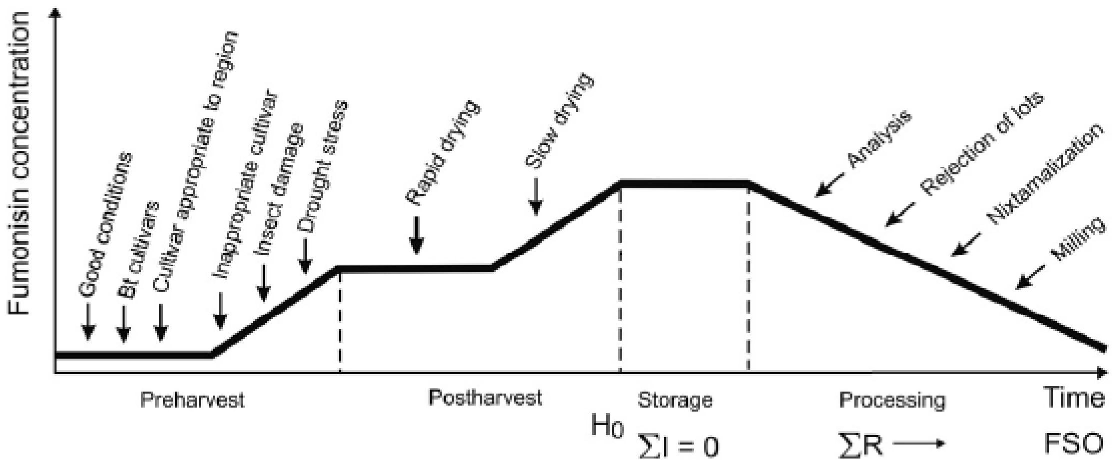

Fig. 7. The time course of fumonisin formation and reduction in maize, with reference to the Food Safety Objective.

Figure 1. The time course of fumonisin formation and reduction in maize, with reference to the Food Safety Objective

CONCLUSIONS

(a) The current Code of Practice for the Prevention and Reduction of Mycotoxin Contamination in Cereals contains specific references to *Fusarium* infection, but in general, the recommendations are those indicated for all mycotoxins during planting, pre-harvest, harvest, storage and transport from storage.

(b) In addition to what is outlined in the Code of Practice mentioned above, biological control and predictive models have been proposed to be used for controlling fumonisin (and other mycotoxins) contamination in cereals, including maize. While most biological control studies have been conducted in laboratory settings, with limited application on a field scale, many studies conducted in the last decade showed the application of statistical models to predict fumonisin contamination in the field.

(c) Predicting models have been developed for specific geographical regions in Europe and Argentina. Although it can be developed and applicable to any region, if data are available, these models need to be tested for a number of years to determine their feasibility, due to the high variability associated with the levels of fumonisins. More research is needed to evaluate if any of the models developed for specific regions can consistently and accurately be applied over other growing regions. Likewise, predictive modelling can be also applied to quantify the fumonisin reduction through certain processing steps in maize.

(d) The Code of Practice also indicates that freshly harvested cereals should be cleaned to remove damaged kernels and other foreign matters. It is essential that sorting and cleaning, within Good Manufacturing Practices (GMP), should also be applied systematically at the industry level before the grain goes for further processing, in order to maintain fumonisin contamination in milling products at safe levels for consumers.

(e) The Code of Practice indicates that Hazard Analysis Critical Control Point (HACCP), a food safety management system that is used to identify and control hazards within the production and processing, should be considered in the future as complementary management system to prevent and reduce mycotoxin levels. A HACCP manual has already been developed by FAO/IAEA.

(f) The FSO framework for fumonisin in maize is an useful risk management tool for coordinating the production process throughout the farm-to fork production chain. Good Agricultural practice (GAP), Good hygiene practices (GHP), GMP and HACCP are the primary tools to achieve FSO, thus FSO should be based on a realistic assessment of what can be achieved through these.

RECOMMENDATIONS

1) The Committee should consider a new work for the revision of the Code of Practice for the Prevention and Reduction of Mycotoxin Contamination in Cereals. This revision should include, among others, the following aspects:

a. When available for a specific region, predicting models may be used by the farmers as an additional tool to prevent and control mycotoxins in cereals, including fumonisins in maize

b. Sorting and cleaning should be considered essential within the GMP procedures and HACCP system to be applied by the industry in order to decrease fumonisin levels in processed products

2) A HACCP manual developed by FAO/IAEA should be reviewed to determine if it can be adopted for the control of fumonisin in maize and other cereals.

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Annex

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