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CODEX ALIMENTARIUS COMMISSION

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World Health Organization

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CODEX COMMITTEE ON CONTAMINANTS IN FOODS

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DISCUSSION PAPER ON THE POSSIBLE REVISION OF THE *CODE OF PRACTICE FOR THE PREVENTION AND REDUCTION OF MYCOTOXIN CONTAMINATION IN CEREALS* (CAC/RCP 51-2003)

(Prepared by the Electronic Working Group chaired by Brazil and co-chaired by the United States of America)

BACKGROUND

1. The 6th Session of the Codex Committee on Contaminants in Foods (CCCF) (March 2012) agreed to develop a discussion paper to identify gaps in the *Code of Practice for the Prevention and Reduction of Mycotoxin Contamination in Cereals* (CAC/RCP 51-2003) (COP) in order to determine the need for a separate code of practice for fumonisins in maize and whether there were any other measures to control fumonisins in this product.[1]

2. At the 7th Session of the Committee (April 2013), the delegation of Brazil, as chair of the Electronic Working Group on Fumonisins in Maize and Maize Products, informed the Committee that, in reviewing the code of practice, it was found that it focused mainly on primary production and that it would be useful to include effective good manufacturing practices such as sorting and cleaning to remove damaged kernels and other foreign matter at the industry level. Predictive models were proposed for the control of mycotoxins, including fumonisins, that could be included in the code of practice which, at the time of its adoption, included a section on hazard analysis and critical control points (HACCP) as a future food safety management system that could be included in the Code.

3. Noting that the Code was adopted ten years ago and that new information is available as raised above, it was proposed to revise the Code to take into account this new information. It was noted that the measures mentioned above were not necessarily specific for fumonisins and that the revision would therefore apply to all mycotoxins. It was also noted that a revision of the general section of the code of practice could have an impact on the annexes, and that the annexes should therefore also be reviewed to ensure consistency with the main Code.

4. The Committee agreed that it was too early to start new work on the revision of the Code and that more information was needed on the nature of the revision. It was therefore agreed to re-establish the EWG, led by Brazil and co-chaired by the United States of America, to further develop the discussion paper and to propose a revision of the Code for consideration by the next session of the Committee.[2] The List of Participants of the EWG is presented in Appendix IV.

INTRODUCTION

5. The *Code of Practice for the Prevention and Reduction of Mycotoxin Contamination in Cereals* (CAC/RCP 51-2003) was developed in an attempt to control and manage mycotoxin contamination worldwide. The Code states the importance of implementing good agricultural practices (GAPs) and good manufacturing practices (GMPs) by producers, indicating the adoption of a complementary management system to be considered in the future i.e. the HACCP system.

6. The Code contains general principles for the reduction of mycotoxins in cereals during planting, pre-harvest, harvest, storage and transport from storage. There are also annexes with additional measures for zearalenone, fumonisins, ochratoxin A and trichothecene.

7. This discussion paper focuses mainly on the aspects not completely covered in the Code.

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1 REP12/CF, para. 92.
2 REP13/CF, paras. 127-132.

8. To prepare this discussion paper, a review of the work published, mostly in the last ten years, on mycotoxins in cereals was performed, and the information summarized in the Background Information presented in Appendix III. The proposed draft Annex on the Prevention and Reduction of Aflatoxins (AFs) and Ochratoxin A (OTA) in Sorghum, currently under consideration by the CCCF (CX/CF 14/8/10) and the Discussion Paper on Aflatoxins in Cereals (CX/CF 14/8/15), were also considered in the review process. In addition, comments submitted by members of the EWG including the recommendations made at the 7th Session of the CCCF were taken into account in the development of the discussion paper.

CONCLUSIONS

9. The EWG agreed that there is a need to revise the *Code of Practice for the Prevention and Reduction of Mycotoxin Contamination in Cereals* to include the knowledge gained in the last 10 years on fungus-plant interaction and mycotoxin production, as shown in the Background Information. This revision should also include farmers and government experience in dealing with mycotoxin issues locally, as provided in the comments of the EWG.

10. The main points identified for revision in this discussion paper are:

- The HACCP system should be incorporated into the Code.
- An Annex on Aflatoxin should be added to the COP. The importance of this mycotoxin in cereals is shown in the documents currently under discussion in the CCCF: Prevention and Reduction of Aflatoxins (AFs) and Ochratoxin A (OTA) in Sorghum (CX/CF 14/8/10) and Aflatoxins in Cereals (CX/CF 14/8/15).
- A section on "Processing" should be added to the general part of the COP and in the respective annexes, as many processes have been shown to reduce mycotoxin content in grain and processed products.
- The use of biological control for mycotoxin control should be included in the COP. Commercial products are currently available to control *Aspergillus flavus* in maize.
- The use of predictive models should be included in the COP. Models are currently available to predict pesticide application and/or harvest time for *Fusarium* mycotoxins.

11. The project document with the proposal for new work is presented in Appendix I.

12. The recommended amendments to the *Code of Practice for the Prevention and Reduction of Mycotoxin Contamination in Cereals, including Annexes on Ochratoxin A, Zearalenone, Fumonisins and Tricothecene* (CAC/RCP 51-2003) is presented in Appendix II. The format of this Appendix is as follows:

- Additional information to the COP is underlined and excluded text is in ~~strikethrough~~.
- In some cases, text was deleted or summarized to avoid repetition.
- Relevant changes are justified.

13 The Committee is invited to consider the conclusions as stated in paragraphs 9-10 in order to determine the need for the revision of the Code and if so to consider the project document in Appendix I. In considering the conclusions, the Committee is kindly invited to take into consideration the *Annex for the prevention and reduction of aflatoxins and ochratoxin A contamination in sorghum* (CX/CF 14/8/10) and the conclusions and recommendations of the discussion paper on aflatoxins in cereals (CX/CF 14/8/15[3]) in order to have a consistent approach to management measures for the control of mycotoxins in cereals.

[3] Working documents for consideration by the 8th Session of the Codex Committee on Contaminants in Foods are available on the Codex website at: http://www.codexalimentarius.org/meetings-reports/en/ or by accessing the ftp-link: ftp://ftp.fao.org/codex/meetings/cccf/cccf8

APPENDIX I

PROJECT DOCUMENT

PROPOSAL FOR NEW WORK ON THE REVISION OF THE "CODE OF PRACTICE FOR THE PREVENTION AND REDUCTION OF MYCOTOXIN CONTAMINATION IN CEREALS, INCLUDING ANNEXES ON OCHRATOXIN A, ZEARALENONE, FUMONISINS, TRICHOTHECENE (DON) AND AFLATOXINS (CAC/RCP 51-2003)"

1. Purpose and Scope of the new work

The purpose of the proposed new work is to provide ~~to~~ member countries, cereal producers and industry guidance on how to prevent and reduce mycotoxin contamination in cereals. This guidance will include the latest developments in GAP and GMP in use worldwide.

2. Relevance and timeliness

The *Code of Practice for the Prevention and Reduction of Mycotoxin Contamination in Cereals including Annexes on Ochratoxin A, Zearalenone, Fumonisins and Trichothecenes* was adopted by the Codex Alimentarius Commission in 2003. Since then, a wide range of research was conducted to understand the fungus-plant interaction, mycotoxin biosynthesis and metabolism and measures for the prevention and reduction of mycotoxin contamination in food, such as use of predictive models and biological control. Hence, a revision of the Code taking into consideration these new developments in science and technology ~~information~~ is necessary. In addition, specific management measures to control aflatoxin contamination in cereals are also necessary and will be addressed in a separate annex to the Code.

3. Main aspects to be covered

Specific measures for the control of aflatoxins and additional measures for the prevention and reduction of mycotoxins in cereals, not currently included in the Code, in order to bring the document in line with current GAPs and GMPs and other relevant methodologies and technologies currently in use and widely applied, such as the use of biological control methods and predictive models.

4. Assessment against the criteria for the establishment of work priorities

Consumer protection from the health point of view, food safety, ensuring fair practices in the food trade and taking into account the needs of developing countries. The new work will provide additional and updated guidance to countries in order to prevent and reduce mycotoxin contamination and consequently minimizing consumer dietary exposure from cereals and cereal-based products thereby improving the overall quality of these products.

5. Relevance to Codex Strategic Goals

The proposed work falls under 3 Codex Strategic Goals of the *Codex Strategic Plan 2014-2019*:

Goal 1. Establish international food standards that address current and emerging food issues

Mycotoxin contamination in cereals is a safety issue that impacts on public health, food security and trade.

Goal 2. Ensure the application of ~~food~~ risk analysis principles in the development of Codex standards

This work will help in establishing risk management options and strategies to prevent and reduce mycotoxin levels in cereals. After these practices are implemented, new data can be obtained and a new risk analysis can be performed to evaluate the impact of this revision and may also facilitate the establishment of maximum levels for mycotoxins in cereals and cereal-based products.

Goal 4. Implement effective and efficient work management system and practices.

Reviewing and implementing the recommended practices from primary production to industry level can help to control mycotoxin contamination.

6. Information on the relationship between the proposal and other existing Codex documents

The *Code of Practice for the Prevention and Reduction of Mycotoxin in Cereals* is an inclusive document addressing general GAPs and GMPs applying across cereals and includes specific management measures for certain mycotoxins. This Code supports the application of maximum levels for mycotoxins in cereals available in the *General Standard for Contaminants and Toxins in Food and Feed* (CODEX STAN 193-1995). The Code will also complement other relevant Codex texts in existence or under development such as the *Code of Hygienic Practice for Low-Moisture Foods* (Codex Committee on Food Hygiene)

7. Identification of any requirement for and availability of expert scientific advice

Additional scientific advice is not necessary.

8. Identification of any need for technical input to the standard from external bodies

There is no need for additional technical input from external bodies.

9. The proposed timeline for completion of the new work

2014 – Approval of new work by the 37th Session of the Codex Alimentarius Commission (July 2014).

2015 / 2017 – Consideration of the Code at Steps 4 and 7 in the CCCF. This timeframe provides for flexibility to revise the Code thoroughly.

If there is agreement, the Code can be finalized in 2015 or 2016 following the regular procedure namely advancement of the Code at Step 5/8 (omission of Steps 6/7) for adoption by the CAC in 2015 or advancement of the Code at Step 8 for adoption by the CAC in 2016.

2017 – Adoption of the Code by the 40th Session of the CAC.

APPENDIX II

Recommended amendments to the *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)

CODE OF PRACTICE FOR THE PREVENTION AND REDUCTION OF MYCOTOXIN CONTAMINATION IN CEREALS, INCLUDING ANNEXES ON OCHRATOXIN A, ZEARALENONE, FUMONISINS, TRICHOTHECENES AND AFLATOXINS

CAC/RCP 51-2003 (rev 2014)

1. The complete elimination of mycotoxin contaminated commodities is not achievable at this time. The elaboration and acceptance of a General Code of Practice by Codex will provide uniform guidance for all countries to consider in attempting to control and manage contamination by various mycotoxins. In order for this Code of Practice to be effective, it will be necessary for the producers in each country to consider the general principles given in the Code, taking into account their local crops, climate, and agronomic practices~~, before attempting to implement provisions in the Code~~. It is important for producers to realize that Good Agricultural Practices (GAP) represent the primary line of defense against contamination of cereals with mycotoxins, followed by the implementation of Good Manufacturing Practices (GMP) during the handling, storage, ~~processing~~, and distribution of cereals for human food and animal feed. Industry has the role to implement GMP where required, mainly during processing.

2. Where necessary, cereal growers should be trained to follow GAP and maintain close relation with agricultural advisors and governmental authorities to obtain information and advice regarding choice of appropriate plant protection products and cultivars available for their location that would minimize mycotoxin levels in cereal grains.

   Justification: *text brought from Annex 4 (trichothecenes), modified to be more general*

3. The recommendations for the reduction of mycotoxins in cereals ~~are divided into two parts:~~ consider the recommended practices based on GAP and GMP ~~Good Agricultural Practice (GAP) and Good Manufacturing Practice (GMP)~~, in addition to ~~a complementary management system to consider in the future is~~. the Hazard Analysis Critical Control Points (HACCP) or equivalent processes. The implementation of HACCP principles will minimize mycotoxin contamination through applications of preventive control measures to the extent feasible mainly during storage and processing of cereals.

   Justification: *part of the text on previous para. 40*

4. This General Code of Practice contains general principles for the reduction of various mycotoxins in cereals that should be sanctioned by national authorities. National authorities and other organizations should educate producers regarding the environmental factors that promote infection and growth of the toxic fungi, and toxin production in cereal crops at the farm level. Emphasis should be placed on the fact that the planting, preharvest and postharvest strategies for a particular crop will depend on the climatic conditions of that particular year, taking into account the local crops, and traditional production conditions for that particular country or region. There is need to ~~develop~~ make available to producers/handlers/processors quick, affordable and accurate test kits and associated sampling plans that will allow testing of grain shipments without undue disruption of operations. Procedures should be in place to properly handle, through segregation, reconditioning, recall or diversion, cereal crops that may pose a threat to human and/or animal health. National authorities should support research on methods and techniques to prevent fungal contamination in the field and during harvest and storage.

Justification: *Numerous "quick, affordable" test kits are commercially available.*

Planting

5. Consider developing and maintaining a crop rotation schedule to avoid planting the same ~~commodity in a field in two consecutive years~~ crop in the same field, for two consecutive seasons in order to reduce the inoculum in the field. ~~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 to be used in rotation to reduce the inoculum in the field~~.

   Justification: *a more general statement was made to account for aflatoxigenic fungi. The excluded text was included in the specific annexes.*

6. When possible and practical, prepare the seed bed for each new crop by plowing under or by destroying or removing old seed heads, stalks, and other debris that may have served, or may potentially serve as substrates for the growth of mycotoxin-producing fungi. In areas that are vulnerable to erosion, no-till practices may be required in the interests of soil conservation.

7. Utilize the results of soil tests to determine if there is need to apply fertilizer and/or soil conditioners to assure adequate soil pH and plant nutrition to avoid plant stress, especially during seed development.

8. When available, grow seed varieties developed for resistance to seed-infecting fungi and insect pests. Only seed varieties recommended for use in a particular area of a country should be planted in that particular area.

9. As far as practical, crop planting should be timed to avoid high temperature and drought stress during the period of seed development and maturation. Predictive models, when available, could be used as a tool to plan for the best planting period.

   Justification: *as it was already identified previously, predictive models to control fungal infection, mainly Fusarium, is already in place in many countries for a variety of crops (see background information)*

10. ~~Avoid overcrowding of plants~~ Ensure appropriate density of planting by maintaining the recommended row and intra- plant spacing for the species/varieties grown. Information concerning plant spacing may be provided by seed companies or authorized bodies.

Preharvest

11. Where possible, minimize insect damage and fungal infection in the vicinity of the crop by proper use of registered insecticides, fungicides and other appropriate practices within an integrated pest management program. Predictive models could be used to plan the best application time for pesticides.

    Justification: *information supported by the literature (see background information)*

12. Control weeds in the crop by ~~use of~~ using mechanical methods, ~~or by use of~~ registered herbicides or other safe and suitable weed eradication practices.

13. Minimize mechanical damage to plants during cultivation.

14. If irrigation is used, ensure that it is applied evenly and that all plants in the field have an adequate supply of water. Irrigation is a valuable method of reducing plant stress in some growing situations. Excess precipitation during anthesis (flowering) makes conditions favorable 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.

15. 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.

16. Before harvest time, make sure that all equipment, which is to be used for harvesting and storage of crops, is functional. A breakdown during this critical period may cause grain quality losses and enhance mycotoxin formation. Keep important spare parts available on the farm to minimize time loss from repairs. Make sure that the equipment needed for moisture content measurements is available and calibrated.

Harvest

17. Containers (e.g., wagons, trucks) to be used for collecting and transporting the harvested grain from the field to drying facilities, and to storage facilities after drying, should be clean, dry and free of old grain, grain dust, insects and visible fungal growth before use and re-use.

18. As far as possible, avoid mechanical damage to the grain and avoid contact with soil during the harvesting operation. Steps should be taken to minimize the spread of infected seed heads, chaff, stalks, and debris onto the ground where spores may inoculate future crops.

19. During the harvesting operation, the moisture content should be determined in several spots of each load of the harvested grain since the moisture content may vary considerably within the same field.

20. Immediately after harvest, determine moisture content of the lot, and if necessary, ~~where applicable,~~ dry the crop to the moisture content recommended for storage (generally less than 15%). This is important to prevent further growth of fungal species that may be present on fresh grains, especially *Fusarium* species.~~of that crop~~. ~~Samples taken for moisture measurements should be as representative of the lot as possible. To reduce the variation of moisture content within a lot, the grain may be moved to another facility (or silo) after the drying process~~.

~~20. 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 This is necessary to prevent further growth of a number of fungal species that may be present on fresh grains, especially Fusarium species~~.

    Justification: *paragraphs 19 and 20 were merged and summarized*

21. 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 and optical sorting, may remove ~~some~~ broken kernels that are susceptible to infection ~~infected kernels~~. ~~More research is needed to develop practical procedures for separating symptomless infected kernels from those that are not infected~~.

    Justification: *Many studies were conducted in the topic in the last 10 years. Additionally, "more research is needed" is a general statement that could be applied to many other topics*

Storage

22. An integrated pest management program should also be applied during storage. Avoid piling or heaping high-moisture, freshly harvested commodities for more than a few hours prior to drying or threshing to lessen the risk of fungal growth. Sun drying should be done on clean surfaces, not in direct contact with soil, and grains should be protected from rain and dew during this process. ~~as sun drying of some commodities in high humidity may result in fungal infection~~. Aerate the commodities by forced air circulation where required. Flat bed and re-circulating batch driers are adequate for small scale operations while using continuous flow-dryer will suffice for large scale drying for long storage period.

Justification: *include text from the sorghum document*

23. Make sure that the storage facilities include dry, well-vented structures that provide protection from rain, drainage of ground water, protection from entry of rodents, ~~and~~ birds and insects and ~~minimum~~ minimize the impact of temperature fluctuations.

24. Start with high quality and mature grains where possible, which are free from mechanical, insect or moldy damage. Crops to be stored should be dried to safe moisture levels where required, and cooled as quickly as possible after harvest. Minimize the amount of foreign materials and damaged kernels in stored grains. ~~Refer to paragraph 29 to evaluate the use of approved pesticides~~.

25. The mycotoxin level in in-bound and out-bound grain should be monitored when warranted, using appropriate sampling and testing programs.

26. For bagged commodities, ensure that bags are clean, dry and stacked on pallets or incorporate a water impermeable layer between the bags and the floor. Bags that facilitate aeration are preferable for storage.

27. Where possible, aerate the grain by circulation of air through the storage area to maintain proper and uniform temperature levels throughout the storage area. Grain can also be transferred from one storage container to another to promote aeration and disruption of potential hot spots during storage. Check moisture content and temperature in the stored grain at regular intervals during the storage period.

28. Measure the temperature of the stored grain at several fixed time intervals during storage. A temperature rise of 2-3°C may indicate microbial growth and/or insect infestation. Separate the apparently infected portions of the grain and send samples for analysis. When separated, lower the temperature in the remaining grain and aerate. Avoid using infected grain for food or feed production.

29. Use good housekeeping procedures to minimize the levels of insects and fungi in storage facilities. This may include the use of suitable, registered insecticides and fungicides or appropriate alternative methods within an integrated pest management program. Care should be taken to select only those chemicals that will not create a safety concern based on the intended end use of the grains and should be strictly limited.

30. The use of a suitable, approved preservative (e.g., organic acids such as propionic acid) may be beneficial. These acids are effective in killing various fungi and thus prevent the production of mycotoxins in grains intended only for animal feed. The salts of the acids are usually more effective for long-term storage. Care must be taken because these compounds can negatively affect the taste and odor of the grain.

31. Document the harvesting and storage procedures implemented each season by making notes of measurements (e.g., temperature, moisture, and humidity) and any deviation or changes from traditional practices. This information may be very useful for explaining the cause(s) of fungal growth and mycotoxin formation during a particular crop year and help to avoid similar mistakes in the future.

Transport from storage

32. Transport containers should be dry and free of old grain, visible fungal growth, insects and any contaminated material. As necessary, transport containers should be cleaned and disinfected with appropriate substances (should not cause off-odors, flavor or contaminate the grain) before use and re-use and be suitable for the intended cargo. The use of registered fumigants or insecticides may be useful. At unloading, the transport container should be emptied of all cargo and cleaned as appropriate.

33. Shipments of grain should be protected from additional moisture by using covered or airtight containers or tarpaulins. ~~Avoid~~ Minimize temperature fluctuations and measures that may cause condensation to form on the grain, which could lead to local moisture build-up and consequent fungal growth and mycotoxin formation.

34. Avoid insect, bird and rodent infestation during transport by the use of insect-and rodent proof containers or insect and rodent repellent chemical treatments if they are approved for the intended end use of the grain.

Processing

35. Sorting and cleaning are effective processes to remove contaminated grains and reduce mycotoxin content in cereals. Mold infected and/or damaged kernels should be discarded in order to prevent their entry into the food chain and feed manufacturing process.

36. It is important that the cereal lot is tested for mycotoxin content before going into further processing, especially when the risk of mycotoxin contamination is high. Lots containing higher levels should undergo processing that significantly decrease mycotoxin levels to guarantee a safe product to consumers.

37. Brushing, scouring and peeling the grain significantly reduce mycotoxin content, as the outer parts of the kernel contain higher mycotoxin levels.

38. Dry milling processing of grain can reduce the mycotoxin content of milled products used as food ingredients. Wet milling of maize grain isolates most mycotoxins from the starch fraction used as food ingredients.

39. The HACCP system is an important tool to define which steps of the processing should be controlled to minimize the presence of mycotoxins in food.

~~II. A COMPLEMENTARY MANAGEMENT SYSTEM TO CONSIDER IN THE FUTURE~~

~~35. The Hazard Analysis Critical Control Point (HACCP) system is a food safety management system that is used to identify and control hazards within the production and processing system. The general principles of HACCP have been described in several documents~~.

~~36. The HACCP concept is an all-encompassing integrated management system. When properly implemented, this system should result in a reduction of the levels of mycotoxins in many cereal grains. The use of HACCP as a food safety management system has many benefits over other types of management control systems in some segments of the food industry. At farm level, especially in the field, many factors that influence the mycotoxin contamination of cereals are environmentally related, such as weather and insects, and are difficult or impossible to control. In other words, critical control points often do not exist in the field. However, after harvesting, critical control points may be identified for mycotoxins produced by fungi during storage. For example, a critical control point could be at the end of the drying process and one critical limit would be the water content/water activity~~.

~~37. It is recommended that resources be directed to emphasizing Good Agricultural Practices (GAPs) at the preharvest level and Good Manufacturing Practices (GMPs) during the processing and distribution of various products. A HACCP system should be built on sound GAPs and GMPs~~.

~~38. It is also recommended that before further consideration is given to the HACCP system, reference should be made to the Codex Annex to CAC/RCP 1-1969, Rev. 4 (2003) "Hazard Analysis and Critical Control Point (HACCP) System and Guidelines for its Management"~~.

~~39. Consideration should also be given to a HACCP manual for mycotoxin control recently published by FAO/IAEA~~.

~~40. At the Third International Conference on Mycotoxins, which took place in Tunisia in March 1999, one of the general recommendations was that integrated mycotoxin control programs should incorporate HACCP principles in the control of risks associated with mycotoxin contamination of foods and feeds.4 The implementation of HACCP principles will minimize mycotoxin contamination through applications of preventive controls to the extent feasible in the production, handling, storage and processing of each cereal crop~~.

Justification: *not necessary, it is included in Paragraph 2*

ANNEX 1

PREVENTION AND REDUCTION OF CONTAMINATION BY ZEARALENONE IN CEREAL GRAINS

RECOMMENDED PRACTICES BASED ON GOOD AGRICULTURAL PRACTICE (GAP) AND GOOD MANUFACTURING PRACTICE (GMP)

1. Good Agricultural Practice includes methods to reduce *Fusarium* infection and zearalenone contamination of cereals in the field and during planting, harvest, storage, transport and processing.

Planting

2. Refer to paragraphs 5-10 in the General Code of Practice for the Prevention and Reduction of Mycotoxin Contamination in Cereals.

Preharvest

3. Refer to paragraphs 11-16 in the General Code of Practice.

4. The establishment of *Fusarium* infection in cereal heads during flowering ~~should~~ may need to be monitored before harvest by sampling, and determination of infection by standard microbiological methods. Also, mycotoxin content in representative preharvest samples ~~should~~ may need to be determined. Utilization of the crop should be based on prevalence of infection and mycotoxin content of the grain.

5. Zearalenone risk in wheat increases with preharvest rainfall. Predictive modelling may be useful to plan to harvest grain before wet weather conditions may emerge.

6. 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 that are not hosts to *Fusarium* species should to be used in rotation to reduce the inoculum in the field.

   Justification: *previously in the General Code (para 5, previous 4)*

Harvest

7. Refer to paragraphs 17-21 in the General Code of Practice.

Storage

8. Refer to paragraphs 22-31 in the General Code of Practice.

Transport from storage

9. Refer to paragraphs 32-34 in the General Code of Practice.

Processing

10. Refer to paragraphs 35-39 in the General Code of Practice.

~~**10.** Small, shriveled grain may contain more zearalenone than healthy normal grain. Winnowing grains at harvest or later will remove shriveled grain~~.

Justification: *winnowing is a kind of sorting and cleaning, which are included in para. 35.*

~~Zearalenone management system based on hazard analysis critical control point system (HACCP)~~

~~9. Refer to paragraphs 35-40 in the General Code of Practice~~.

Justification: *the HACCP section was removed*

ANNEX 2

PREVENTION AND REDUCTION OF CONTAMINATION BY FUMONISINS IN CEREAL GRAINS

RECOMMENDED PRACTICES BASED ON GOOD AGRICULTURAL PRACTICES (GAP) AND GOOD MANUFACTURING PRACTICE (GMP)

1. Good Agricultural Practice includes methods to reduce *Fusarium* infection and fumonisin contamination of cereals in the field and during planting, harvest, storage, transport and processing.

Planting

2. Refer to paragraphs 5-10 in the General Code of Practice.

Preharvest

3. Refer to paragraphs 11-16 in the General Code of Practice.

Harvest

4. Refer to paragraphs 17-21 in the General Code of Practice.

5. 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. Predictive models may be used for planning the best harvest time.

6. 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 that are not hosts to *Fusarium* species should to be used in rotation to reduce the inoculum in the field.

Justification: *previously in the General Code (para 5, previous 4)*

Storage

7. Refer to paragraphs 22-31 in the General Code of Practice.

Transport from storage

8. Refer to paragraphs 32-34 in the General Code of Practice.

Processing

9. Refer to paragraphs 35-39 in the General Code of Practice.

10. Nixtamalization, a process that involves boiling and soaking maize in a solution of calcium hydroxide, may reduce fumonisin levels in tortillas and other maize products,

11. Extrusion of maize may decrease fumonisin levels, however part of it is bound to proteins, sugars or other compounds in food matrices

~~Fumonisins management system based on hazard analysis critical control point system (HACCP)~~

~~8. Refer to paragraphs 35-40 in the General Code concerning HACCP.~~

Justification: *the HACCP section was removed*

ANNEX 3

PREVENTION AND REDUCTION OF CONTAMINATION BY OCHRATOXIN A IN CEREAL GRAINS

RECOMMENDED PRACTICES BASED ON GOOD AGRICULTURAL PRACTICES (GAP) AND GOOD MANUFACTURING PRACTICE (GMP)

1. Good Agricultural Practice includes methods to reduce fungal infection and ochratoxin A contamination of cereals in the field and during planting, harvest, storage, transport and processing.

Planting

2. Refer to paragraphs 5-10 in the General Code of Practice.

Preharvest

3. Refer to paragraphs 11-16 in the General Code of Practice.

4. Factors during preharvest that may affect levels of ochratoxin A in harvested grains include frost damage, presence of competitive fungi, excessive rainfall and drought stress.

Harvest

5. Refer to paragraphs 17-21 in the General Code of Practice.

Preservation

6. Ochratoxin is mostly a storage mycotoxin. Grain should be allowed to dry as much as possible before harvest consistent with local environment and crop conditions. If unable to harvest the grain when it has a water activity below 0.70, then dry the grain to a moisture content corresponding to a water activity of less than 0.70 (less than 14% moisture content in small grain) as quickly as possible. To avoid ochratoxin A formation, start the drying process immediately after harvest ~~and preferably use heated-air drying~~. In the temperate climate region, when intermediate or buffer storage is necessary because of low drying capacity, make sure that the moisture content is less than 16%, that the buffer storage time is less than 10 days, and the temperature is less than 20 °C.

Storage

6. Refer to paragraphs 22-31 in the General Code of Practice.

Transport from storage

7. Refer to paragraphs 32-34 in the General Code of Practice.

Processing

8. Refer to paragraphs 34-39 in the General Code of Practice.

~~Ochratoxin a management system based on hazard analysis critical control points (HACCP)~~

~~9. Refer to paragraphs 35-40 in the General Code of Practice.~~

Justification: *the HACCP section was removed*

ANNEX 4

PREVENTION AND REDUCTION OF CONTAMINATION BY TRICHOTHECENES IN CEREAL GRAINS

RECOMMENDED PRACTICES BASED ON GOOD AGRICULTURAL PRACTICES (GAP) AND GOOD MANUFACTURING PRACTICE (GMP)

1. Good Agricultural Practice includes methods to reduce *Fusarium* infection and trichothecene contamination of cereals in the field and during planting, harvest, storage, transport and processing.

Planting

2. Refer to paragraphs 5-10 in the General Code of Practice.

Preharvest

3. Refer to paragraphs 11-16 in the General Code of Practice.

~~4. Do not permit mature grains to remain in the field for extended periods of time, particularly in cold, wet weather. T-2 and HT-2 toxins are not usually found in grains at harvest, but can result from grains that are water-damaged in the field or grains that become wet at harvest or during storage.~~

4. ~~Refer to paragraph 4 in Annex 1.~~ Predicting models may assist producers in decisions on whether or not to apply fungicides. The establishment of *Fusarium* infection in cereal heads during flowering may need to be monitored before harvest by sampling and determination of infection by standard microbiological methods. Also, mycotoxin content in representative preharvest samples may need to be determined. Utilization of the crop should be based on prevalence of infection and mycotoxin content of the grain.

Justification: *first sentence was added and the indicated paragraph was repeated here*

~~6. Cereal growers should maintain close relations with local cereal trade groups. Such groups should be important sources of information and advice regarding choice of appropriate plan protection products, cultivars and strains that will take into account those resistant to *Fusarium* and are available for their location~~.

Justification: this issue was included in *para* 2 of the General Code of Practice.

Harvest

5. Refer to paragraphs 17-21 in the General Code of Practice.

6. Do not permit mature grains to remain in the field for extended periods of time, particularly in cold, wet weather. T-2 and HT-2 toxins are not usually found in grains at harvest, but can result from grains that are water-damaged in the field or grains that become wet at harvest or during storage.

Justification: *more appropriated in the harvest section*

Storage

7. Refer to paragraphs 22-31 in the General Code of Practice.

Transport from storage

8. Refer to paragraphs 32-34 in the General Code of Practice.

Processing

9. Refer to paragraphs 35-39 in the General Code of Practice.

10. Extrusion may reduce trichothecene levels in processed products, especially of deoxynivalenol.

~~Thricothecene management system based on hazard analysis critical control points (HACCP)~~

~~9. Refer to paragraphs 35-40 in the General Code of Practice~~.

Justification: *the HACCP section was removed*

ANNEX 5

PREVENTION AND REDUCTION OF CONTAMINATION BY AFLATOXINS IN CEREAL GRAINS

RECOMMENDED PRACTICES BASED ON GOOD AGRICULTURAL PRACTICE (GAP) AND GOOD MANUFACTURING PRACTICE (GMP)

1. Good Agricultural Practice includes methods to reduce *Aspergillus* infection and aflatoxin contamination of cereals in the field and during planting, harvest, storage, transport and processing.

Planting

2. Refer to paragraphs 5-10 in the General Code of Practice.

Preharvest

3. Refer to paragraphs 11-16 in the General Code of Practice.

4. Biological control can be used for aflatoxins, but the product must be approved, safe, and cost-effective towards the targeted plant pathogen.

Harvest

5. Refer to paragraphs 17-21 in the General Code of Practice.

Storage

6. Refer to paragraphs 22-31 in the General Code of Practice.

7. The best way to control aflatoxins in cereals is the prevention of its formation during the storage. The time between harvest and drying for storage should be minimized.

Transport from storage

8. Refer to paragraphs 32-34 in the General Code of Practice.

Processing

9. Refer to paragraphs 35-39 in the General Code of Practice.

ENGLISH ONLY

APPENDIX III

BACKGROUND INFORMATION

MYCOTOXIN PRODUCTION IN CEREALS

1. The main mycotoxins of significance in cereals are aflatoxins, fumonisins, ochratoxin A, deoxynivalenol and zearalenone, which are produced by species of fungi from the common genera Aspergillus, Penicillium and Fusarium. These fungi may either grow on the crop or invade the crop after harvest, producing toxins during drying and storage. The incidence of mycotoxins depends on a wide variety of agronomic and climate conditions, and on whether a particular cultivar is grown within the area to which it is adapted. The conditions for fungi growth and mycotoxins production are shown on Table 1.

Table 1. Minimal conditions for fungi growth and mycotoxin production by *Aspergillus flavus*, F*usarium* sp and *Penicillium verrucosum*

Fungi	Mycotoxin	Fungi growth				Mycotoxin production		Reference
		T opt (°C)	T min (°C)	T max (°C)	Aw	Aw	T (°C)	
Aspergillus flavus	Aflatoxins	33	10-12	43-48	0.82[a]	0.82	13-37	Pitt and Hocking, 2009
Fusarium graminearium	Zearalenone	24-26			0.90[b]	0.95-0.97	12-28	Jimenez et al., 1996
Fusarium graminearium	Deoxyniva-lenol					0.95-0.995	25	Pitt and Hocking, 2009
Fusarium graminearium	Nivalenol					0.96-0.98	20	Llorens et al., 2004
Fusarium proliferatum	Fumonisin					0.93-0.976[c]		Samapundo et al. 2007
Fusarium verticillioides	Fumonisin	25	2.5-5	32-37	0.87[a]	0.92		Pitt and Hocking, 2009
Penicillium verrucosum	Ocratoxin A	20	0	31	0.80	0.86		Pitt and Hocking, 2009

[a] at 25°C, [b] at 20°C, [c] at 10-20% O_2

2. A fungus may be associated with a plant during the plant growth (pre-harvest) as a commensal or a pathogen, or have no association with the plant, in which case invasion of a crop and mycotoxin production occur post-harvest. Thus, the control of pre-harvest toxin formation must be based on entirely different strategies from the control of toxins which occur post-harvest (Pitt, 2006).

3. The control of pre-harvest mycotoxin formation is more difficult than post-harvest. Uncontrollable factors such as crop, climate and environmental dictate whether the fungi grow and whether mycotoxins are likely to be formed. Two very important generalizations can be made. First, growth of these plant associated fungi occurs only in specific crops, where there is a definite plant-fungus association. Second, the fungi that produce these toxins grow only at high water activity, so these toxins which are formed pre-harvest are rarely formed during storage (Pitt, 2006). The control of post-harvest toxin is basically a food technology problem and could be controlled by implementing GAPs and GMPs.

PREVENTION AND CONTROL OF TOXINS FORMED DURING PRE-HARVEST

4. The pre-harvest control of mycotoxin production depends on the fungi and the associated crop. Table 2 shows the main risk management strategies for the major mycotoxins in pre-harvest.

Table 2. Risk management strategies for the major mycotoxins in cereals during pre-harvest.

Aflatoxins	Fumonisins	Ochratoxin A	Deoxynivalenol and zearalenone
• GAP • Developing drought-resistant cultivars • Biological control • Predictive models • Timely harvesting • Breeding for host plant resistance	• GAP • Ensuring that cultivars are adapted to local environments • Breeding for insect resistance • Breeding for host plant resistance • Transgenic Bt maize • Predictive models • Timely harvesting	• GAP • Timely harvesting	• GAP • Breeding for host plant resistance • Using cultivars that mature over a range of dates • Transgenic Bt maize • Using fungicides at anthesis or silking • Predictive models

Adapted from Pitt et al. (2012).

Good Agricultural Practice (GAP)

5. GAP involves good farm management, in general maintaining healthy crops and sustainable agriculture together with other activities (FAO, 2002):

   (i) Planting with optimal row and seed spacing for local conditions, especially water availability, to reduce plant stress;

   (ii) Maintaining adequate water supplies, by irrigation where practicable;

   (iii) Reducing erosion by contouring, ditching, or hedging;

   (iv) Controlling weeds, and mulching crops to reduce moisture stress;

   (v) Controlling insects that damage developing grains or nuts and permit entry of the fungi that produce mycotoxins;

   (vi) Rotation crops to reduce insect infestation and fungal infection, which are exacerbated by monoculture;

   (vii) Applying fertilizers at appropriate times and concentrations, to benefit the crop but limit run-off of nutrients such as nitrogen and phosphorus;

   (viii) Harvesting crops at or before full maturity, because over mature crops are liable to increase risk from insect damage and water stress and hence mycotoxin production;

   (ix) Drying crops rapidly and completely, as soon as possible after harvest; and

   (x) Maintaining good storage conditions on the farm (storage facilities should be soundly constructed to prevent water ingress, with raised floors to prevent moisture migration from soil; properly dried crops should be stored in closely woven sacks that permit air exchange; and rodents and insects should be controlled)

Conventional and non-conventional breeding for host plant resistance

Aspergillus and aflatoxin resistance

6. Commercial maize hybrids have been evaluated for inherent insect resistance since the early 1970's as well as for reduced aflatoxin levels from *Aspergillusflavus* natural field infection, and researchers have found significant differences in aflatoxin levels among hybrids samples from non-infested plots (Widstrom, et. al, 1978).

7. Cultivars Mo20W x Teosinte and 'Ibadan B' were resistant to maize ear infesting insects compared to control maize hybrids (Barry, et. al., 1991). The specific kernel resistance mechanisms were examined in kernels from maize populations MAS:gk and MAS:pw,nf, resistant to post-harvest aflatoxin contamination by *A. flavus* as well as fungal growth (Brown, et. al., 1993). Only autoclaving, crushing or wounding of the embryo caused a loss of aflatoxin accumulation resistance, and it was concluded that the metabolic activities of the living embryo conveyed the resistance.

8. Plant antifungal proteins were identified in the seeds of maize and other grains (Vigers, et, al., 1991). In a later study inbreds Tex6, Y7, and Mp420, and the F1 hybrids with these inbreds were highly and consistently resistant to *Aspergillus* ear rot, kernel infection and production of aflatoxins (Campbell, 1995). It was determined that the resistance seen in the inbreds in addition to other resistant inbreds, was likely inherited and therefore part of the plant genome. In another experiment, aflatoxin resistant maize genotypes were compared to drought tolerant genotypes as to cuticle and wax layers in the kernel (Tubajika, 2001). The results suggested that the pericarp wax layer in the kernels imparted resistance to *A. flavus* infection as this genotype had very high levels according to microscopic observations.

9. Naidoo et. al. (2002) found the inbreds Tex6 and Oh516 had the lowest levels of aflatoxins and were most resistant to ear rot. The determination of the dominant alleles in inbred MI82 resistant to *A. flavus* and aflatoxin accumulation possibly transferrable to commercial inbreds showed to be promising (Maupin, et. al., 2003).

10. Present-day breeding endeavors concerning the development of aflatoxin-resistant maize lines have resulted in several germplasm releases. In 2008, the IITA-SRRC collaborative breeding program released TZAR 101–106, resulting from a combination of African and southern-adapted US lines (Menkir et al. 2008). The inbred maize line GT-603 was released in 2011, after having originated from GT-MAS:gk (Guo et al. 2011), while Mp-718 and Mp-719 were issued as southern acclimatized resistant lines which unlike the earlier Mp lines, are both shorter and earlier (Scully et al. 2012; Williams & Windham 2012). These lines are being studied in hybrid combinations through the SERAT program (Scully et al. 2012). Unfortunately at this time these maize lines are not commercially viable; however they do offer a source of invaluable resistance genes and can be utilized for the development of host resistant approaches to eliminate aflatoxin contamination (Brown, et. al, 2013).

Fusarium and fumonisin resistance

11. In the early 1990's, work on a sweet maize line resistant to *F. verticillioides* kernel infection, was being conducted to determine genetic factors as the potential mechanism of resistance specifically examining maternal tissues such as the silk, pericarp and closing layer (Headrick, 1991). The field inoculation study determined that silk actively growing after pollination inhibited *F. verticillioides* from infecting the kernel and indicated the potential use of this inbred as the possible parent for use in seed production. In 2001, maize genotypes GT-MAS:gk and MI82 known for *A. flavus* infection and aflatoxin contamination were examined for resistance to *F. verticillioides* growth (Brown, et. al., 2001). It was determined that non-wounded kernels of the resistant hybrid were less susceptible to *F. verticillioides* infection.

12. Resistance to fumonisin accumulation and Fusarium ear rot in maize was studied, with a number of dominant genes determined which conveyed resistance with the potential to transfer these alleles to commercial hybrids (Clements, et. al., 2004; Alexander et al., 2009). Lanubile et al. (2010) found that in the maize resistant line CO441, defense related genes assayed from *F. verticillioides* (b-tubulin 2 and FUM21) were transcribed at high amounts before contamination and supplied rudimentary protecting against the fungus. The FvMK1 mitogen-activated protein kinase gene was identified in *F. verticillioides*, which regulates fungal growth, conidia production, and pathogenesis as well as lowers the action of the FUM1 and FUM8 fumonisin synthesis genes (Zhang et al., 2011).

13. Santiago, et al. (2013) evaluated a large number of maize inbred line kernels inoculation with *Fusariumverticillioides* for Fusarium ear rot and fumonisin accumulation. The highest levels of resistance to Fusarium ear rot and fumonisin accumulation were found in sixty-one of the maize inbreds, which differed in kernel color, use, kernel type and heterotic group. Many of these maize inbreds can be employed to enhance resistance to *F. verticillioides* infection and fumonisin accumulation by performing crosses between the most resistant maize inbreds of each subgroup.

Fusarium, Aspergillus, fumonisin and aflatoxin combined resistance

14. In a multi-lab study, highly resistant maize hybrid lines GT-MAS:gk and Mp420 were screened for proteins conveying antifungal activity against both *A. flavus* and *F. verticillioides* during germination of maize kernels (Guo, et. al., 1997). Higher concentrations were found in the germinated kernels compared to controls, and the 22kDa zeamatin-like protein was present as well as two ribosome inactivating proteins (RIP), of 11-kDa and 9-kDa in size. In the non-germinated kernels, a 32-kDa proRIP-like form and an 18kDa peptide were found. When purified zeamatin and RIP were tested on *A. flavus*, growth of the fungus was inhibited, and plant extracts from germinated kernels inhibited both *A. flavus* and *F. verticillioides*. In another similar study on seeds from maize inbred Tex6, a >100 kDa protein was found to inhibit aflatoxin biosynthesis, and a 28-kDa protein similar to pathogenesis-related proteins-5 (PRs), which is very similar to thaumatin, was determined to inhibit growth (Huang, et. al., 1997). Dry kernel extracts were tested for antifungal activity from resistant populations T115, CI2, Tex-6, and MI82 and were found to contain a 14-kDa protein which was determined to both inhibit *A. flavus* growth and produce trypsin activity causing the spore to rupture and hyphae to develop abnormally (Chen, et, al., 1998). This same maize trypsin inhibitor was found to be an effective inhibitor of both *A. flavus* and *F. verticillioides* fungi simultaneously (Chen, 1999). Five additional proteins were determined to be associated with resistance to aflatoxin production in resistant maize populations GT-MAS:gk, CI2, T115, MP420, and Tex6 that are also stress-related proteins (Chen, et. al., 2001).

15. Twenty four recombinant inbred lines demonstrating the highest mean fumonisin concentration and 24 lines showing the lowest mean fumonisin concentration were selected for further evaluation were inoculated with *F. verticillioides* and *F. proliferatum* or with *A. flavus* in replicated trials. The maize inbred groups of low-fumonisin accumulation were significantly lower in fumonisin and aflatoxin levels as well as Fusarium and Aspergillus ear rots (Robertson-Hoyt, et al, 2007)

16. More recently, 20 inbreds were tested for *F. verticillioides* and *A. flavus* resistance and indicated that inbreds with aflatoxin resistance may be good sources for breeding for fumonisin resistance (Henry et al. 2009). High aflatoxin and fumonisin resistance were demonstrated in the inbreds Mp715 and MP 717. Maize inbred Mp313E revealed resistance against fumonisins only (Williams & Windham, 2009).

Chemical control

17. For some crops, infection can be reduced through the use of fungicides or insecticides. Insecticides can be partially effective only for those infections that are associated with insect injury. Fungicides are widely used in wheat to prevent infection by *Fusarium graminearum* and other fungi that cause Fusarium head blight. Gauging the need for fungicides and optimizing the timing of applications has been greatly improved through the development of risk-assessment models, which are available on-line for many wheat-growing areas (http://www.wheatscab.psu.edu/). Properly timed fungicide applications can reduce infection and mycotoxin contamination, but disease control does not always result in reduced mycotoxin contamination (Schmale and Munkvold, 2013)

Biological control

18. Many biocontrol agents were first identified through *in vitro* inhibition tests, but there is not always a correlation between in vitro inhibition tests and field performance of biocontrol agents (Fravel, 2005). This difference is usually explained by various environmental conditions which may affect the antagonist performance (Cotty; Mellon, 2006) or laboratory conditions may artificially favour the antagonist (Weller, 1988). It is increasingly clear the trend and need of inserting the biocontrol agents in an integrated pest management scheme (Medeiros et al., 2012).

Aflatoxins

19. The *A. flavus* biological control strategy involves the application of an atoxigenic strain of *A. flavus* to the soil surface, which then colonizes the crop and out-competes the existing toxigenic strains (Schmale and Munkvold, 2013). Usually the selected strain is introduced to the field on a carrier substrate that permits growth of the fungus with consequent production of high numbers of spores (Dorner et al. 1999; Taniwaki & Pitt, 2013).

20. Depending on climatic factors and concentration of toxigenic spores in a given field, aflatoxin reductions may range widely. Field experiments in different crops have demonstrated significant reductions in the aflatoxin contamination (70-90%) (Dorner, 2004; Dorner, 2008; Dorner, 2009; Probst et al., 2011). Accinelli et al. (2012) applied bioplastic granules inoculated with atoxigenic strains of *A. flavus* on the soil surface of corn crops, achieving a reduction of aflatoxin contamination by 59-92%.

21. Table 3 shows the two commercially available biocontrol products for *Aspergillus* in corn: Afla-guard® and *Aspergillus flavus* AF36. Both products contain naturally occurring, nontoxigenic strains of *Aspergillus flavus.* Afla-guard® is recommended for AFs control in corn (field, sweet and popcorn) and peanuts, being applied at the first sign of corn tasseling and before active silking. *Aspergillus flavus* AF36 is for use in corn, cotton and pistachio, and its application in corn occurs from the 7 leaf stage (V7) until silking. Currently, no other commercial products are available for other cereals.

Table 3. Biocontrol products developed for control of postharvest diseases and aflatoxin

Product	Microbial agent	Food commodity	Manufacturer/distributor
Aspergillus flavus AF36	*A. flavus* strain AF36	Corn, cotton and pistachio	Arizona Cotton Research/ Protection Council, USA
Afla-guard®	*A. flavus* strain NRRL21882	Peanuts and corn	Syngenta Crop Protection, USA

22. AF36 has been shown to produce cyclopiazonic acid (CPA) in treated maize and peanuts. Chronic toxicity of CPA has not been studied, but recent animal studies show significant harmful effects from short-term exposure to CPA at low doses (King et al., 2011).

Fumonisins

23. For Fusarium head blight, microorganisms that are antagonistic to the *Fusarium* pathogens are applied to the wheat heads. Some reports showed that production of FB1 was reduced up to 63.2%,FB2 up to 43.4% and deoxynivalenol and zearalenone up to 92% and 87.5%, respectively (Stiles and Bullerman,2002). Nayaka et al. (2010) showed that maize seed treated with suspension of *Trichodermaharzianum* followed by spray treatment with pure *T.harzianum* culture suspension reduced the levels of fumonisins in all maize cultivars by 56.4-85.8%.

24. 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*. Dalié et al. (2012) have shown that*Pediococcus pentosaceus* (strain LOO6) produced some extracellular metabolites (MRS medium) capable of reducing 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.

Ocratoxin A

25. Several bacterial and fungal strains belonging to *Streptococcus, Bifidobacterium, Lactobacillus, Butyribrio, Phenylobacterium, Pleurotus, Saccharomyces, Bacillus* and *Acinetobacter* genera and certain fungi belonging to *Aspergillus*, *Alternaria, Botrytis, Cladosporium, Phaffia, Penicillum and Rhizopus* (*R. stolonifer* and *R. oryzae*) genera, are able to degrade OTA in vitro up to more than 95% (Abunrosa et al, 2006).

Predictive models

26. Predicting models have been developed for specific geographical regions in several countries. 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 mycotoxins.

27. 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.

28. 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 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.

29. FUMAgrain, a model developed in Italy, is based on the pathosystem of *F. verticillioides* and *Ostrinianubilalis* (European corn borer) in maize. The elements of the pathosystem are simulated by three submodels: (i) maize development, (ii) *F. verticillioides* infection and fumonisin synthesis and (iii) European Corn Borer wounding activity on maize grain (Maiorano, *et al.*, 2009). 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.

30. 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 is useful for determining the optimal harvest date while respecting grain safety levels required by the international market.

31. 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.

32. 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.

33. DONcast is a web-based interactive model for DON in wheat, which allowed input of field-specific weather and agronomic variables. The model was developed to assist producers in decisions on whether or not to apply a fungicide, and for grain marketing decisions. The predictions have explained 76% of the variability in DON using a database from 1996 to 2003 (Hooker et al., 2002; Schaafsma & Hooker, 2006).

34. These models must be tested and validated in many areas, not only to make the models more widely useful but also to test their robustness to climate variation. 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. As with all predictive models, these require reliable climate and agronomic data and some sophisticated mathematics (Schaafsma & Hooker, 2006).

STORAGE AND TRANSPORT

35. Before going to storage, broken or cracked kernels and foreign matter should be removed from the lot. Broken kernels are more likely to be contaminated or to get contaminated during storage than sound kernels. Foreign material may restrict air movement through the grain mass leading to temperature and moisture problems that may favor storage mould development (Sweets, 2013). It is important that the quality of the grain is monitored and that grain damage and microbial spoilage are minimised as far as possible, in particular with respect to mould growth and mycotoxin production (Eeckhout et al, 2013).

36. General and specific good hygiene and quality control measures should be taken to avoid the spread of inoculum by contaminated trailers, augers, grain stores and all kind of equipment that have not been cleaned and contain leftover contaminated grain from the previous harvest. A rigorous hygiene programme during harvest, storage and transport is hence of great importance (Eeckhout et al, 2013).

37. To avoid mycotoxin contamination and dispersal of mycotoxin-producing moulds, farmers and contractors should be reminded of their obligation to keep their agricultural trailer or truck clean, both at the inside and outside. Hygienic rules should be respected also at the collection or storage facility and the surroundings of the collection facilities (including lawns, concrete areas, pits, etc.) should be properly maintained. Rain and run-off water can drain well. Traps for rodents are placed in the areas surrounding grain storage and waste storage locations (Eeckhout et al, 2013).

38. During storage, the critical points to maintain include regular and accurate moisture and temperature determination and efficient and prompt aeration and drying conditions. Wheat, barley and oats should be kept at 14-15%, maize at 14% and rice at 13-14% of moisture content (Magan & Aldred, 2007; Kabak, et al., 2006; Magan et al., 2003).

39. Giorni et al. (2008) tested the potential of using modified atmospheres (25.0–75.0% CO_2) to control *A. flavus* development and aflatoxin B1 production on maize grain post-harvest. The populations of *A. flavus* were significantly lower with 25 and 75% CO_2 in the atmosphere and all treatments with CO_2 were able to reduce toxin production (57.0-98.0%).

PROCESSING

40. Good manufacturing practice (GMP) is often used to reduce mycotoxin contamination in cereal grains, and includes practices that prevent fungal growth and hence reduce or remove mycotoxin contamination after harvesting and drying. The mycotoxin content and the processing effect on their levels may define the destination of the cereal lots. If the levels do not comply with the grain maximum level, the lot can be directed to specific operational targets for food/feed industry (Cheli et al., 2013; Pitt et al., 2013).

41. The various food processes that may have effects on mycotoxins include sorting, trimming, cleaning, milling, brewing, cooking, baking, frying, roasting, canning, flaking, alkaline cooking, nixtamalization, and extrusion. Most of the food processes have variable effects on mycotoxins, with those that use high temperatures having the greatest effects (Bullerman and Bianchini, 2007). The effects of these practices depend on the crop and mycotoxin and are discussed in details in this document. Food processes that could affect each toxin are summarized in Table 2.

Table 2. Processing that may affect the mycotoxin content in food

Mycotoxin	Processing that may affect the mycotoxin content
Aflatoxin	Sorting, toasting, nixtamalization, chemical treatments, sorbents (for feed), wet milling
Fumoninis	Sorting, nixtamalization, extrusion, flaking, roasting, wet milling
Zearalenone	Sorting, chemical treatment, nixtamalization, extrusion, wet milling
Deoxynivalenol	Sorting, extrusion, wet milling
Ochratoxin A	Sorting, autoclaving, extrusion, roasting

Aflatoxins

42. In general, it is very difficult to decrease aflatoxin levels since they are heat-resistant (up to 260ºC) and soluble in intermediate polar solvents (Hwana and Lee, 2006). Heat treatment under moist conditions is more efficient, but the reduction is affected by the matrix, the binomial time-temperature and products formulation. Reduction of aflatoxin ranges from 28 to 84% in corn products and from 30 to 60% in wheat products. Toasting promotes about 40% reduction in the levels of aflatoxins (Cheli, et al., 2013; Hwang & Lee, 2006).

43. Nixtamalization is a process for making masa for tortillas and other maize products involving boiling and soaking maize in a solution of calcium hydroxide. The process may reduce the content of AFs by 52 to 84%, the aflatoxins G1 and G2 being the most susceptible and aflatoxin B1 the most resistant (Arriola et al. 1988). Another chemical treatment is the application of sodium hydrosulphite associated with heat treatment or pressure, achieving a reduction of 70% of the aflatoxin levels (JALILI & JINAP, 2012).

44. Wet milling of maize for starch production was found to achieve a reduction of aflatoxin levels of 99 – 99.8%. (Yahl et al, 1971; Bennett & Anderson, 1978; Romer, 1984).

45. The effect of citric acid concentration and the moisture content on aflatoxin degradation on sorghum samples was studied during extrusion-cooking milled sorghum, the reduction ranged from 17 to 92% (Méndez-Albores et al. 2009).

46. Soaking of unpeeled rice during the parboiling process makes water to migrate to inner portions of the grain, carrying-over water-soluble compounds. Coelho et al (1999) demonstrated that 32% of AFB1, 44% of AFB2, 36% of AFG1 and 22% of AFG2 migrated into the inner parts of the grain after 6 hours of soaking and autoclaving during 30 minutes before the processing (Coelho et al.1999).

47. Aly and Hathout (2011) concluded that acid hydrolysis of vegetal protein is a suitable method for decontamination of aflatoxin in highly contaminated grains, specially gluten fractions. Completed degradation occurred in the presence of 5mol/L of HCl after 4h at 110°C).

48. The most prevalent approach for counteracting aflatoxin in the feed industry is to include sorbent materials into the feed, for more or less selective removal of toxins by means of adsorption within the route of the gastrointestinal tract (Hwigg, et al., 2001).

49. The fate of aflatoxins and fumonisins was studied through the traditional processing of naturally contaminated maize in mawe, makume, ogi, akassa, and owo, maize-based foods common in Benin, West Africa (Fandohan et al., 2005). Overall reduction of mycotoxin level was more significant during the preparation of makume (93% reduction of aflatoxins, 87% reduction of fumonisins) and akassa (92% reduction of aflatoxins, 50% reduction of fumonisins). Sorting, winnowing, washing, crushing combined with dehulling of maize grains were the unit operations that appeared very effective in achieving significant mycotoxin removal. Fermentation and cooking showed little effect.

Fumonisins

50. The fate of fumonisin during processing is affected by many factors, including the 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.

51. Sorting and cleaning may lower fumonisin concentration by removal of contaminated material, but does not destroy the mycotoxins. Broken maize kernels contain near 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). Afolabi et al. (2006) proposed the visible sorting of maize grain as a technique to reduce fumonisin levels by subsistence farmers.

52. A study conducted in three commercial maize mills showed that fumonisins are distributed in milling streams approximately according to their occurrence in the maize seed structure (Scudamore & Patel, 2009). The concentrations of mycotoxins found in the grits and flours, which are mostly derived from the endosperm typically, contain the lowest mycotoxin levels and concentrations are further 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.

53. Wet-milling is used to obtain maize starch, germ, gluten, solubles and fiber. In laboratory studies of the fate of fumonisin B_1 and B_2 in wet milling the original fumonisin was distributed among solubles (2 – 15%); fiber (19 - 41%); germ (9 – 22%); and gluten (37 – 42%). No fumonisin was detected in the starch fraction (Bennett et al, 1996).

54. Dry-milling gives rise to the bran (obtained from the removal of pericarp) and the germ, followed by the fractions obtained by decreasing particle size - grits, corn meal and flour (Alexander et al., 1987). 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). 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.

55. The effects of heating on the stability of fumonisins depend on the process, the temperature and heating time. Many studies have reported significant reduction of fumonisin levels occurs during processes at temperatures > 150°C, such as those used for dry or moist maize meal production (Scott & Lawrence, 1995), frying maize chips (Jackson et al., 1997), baking, roasting and alkaline cooking (Castelo et al. 1998, Jackson et al. 1997, Katta et al. 1999) and flaking, cooking and toasting (de Girolamo et al., 2001). In all these studies, fumonisins were analyzed by the traditional method which does not detect the bound (hidden, masked) fumonisins.

56. Fumonisins are quite stable and are not destroyed by moderate heat (Castelo et al. 1998b), but an 80% reduction by heating at higher temperatures has been reported (Visconti et al., 1999). However, caution is required in assessing risk as it has been reported that breakdown products such as 'hydrolysed fumonisin' may be formed and these may be almost as toxic as the parent compound (EMAN, 2006).

57. Baking, frying and extrusion cooking of corn at high temperatures (> 190°C) also reduces fumonisin concentrations in foods, with the amount of reduction achieved depending on cooking time, temperature, recipe, and other factors. However, the chemical fate of fumonisins in baked, fried, and extruded foods is not well understood and it is not known if the reduced concentrations result from thermal decomposition of fumonisins or from their binding to proteins, sugars or other compounds in food matrices (Humpf and Voss, 2004).

58. Becker-Algeri et al (2013) evaluated the effects of thermal treatments on FB1 level on polished (husked white), parboiled and whole grain rice (brown, husked). Conventional cooking reduced the initial natural contamination by 80%, autoclaving artificial contaminated samples did not reduce the levels and dry heated treatment reduced 70%.

59. Bullerman et al. (2008) have shown that N-(deoxy-D-fructos-1-yl) FB1 are extruded with glucose, in addition to hydrolyzed FB1 (HFB1) and N-carboxymethyl FB1. Seefelder et al (2003) have shown that FB1 and HFB1 are able to bind to polysaccharides and proteins via their two tricarballylic acid side chains. Currently, analytical methods to detect those bound fumonisins in food matrix are available

60. Voss et al (2006) evaluated the toxicity of maize grits spiked with FB1 extruded with 10% glucose fed to rats. With one exception, the fumonisin B1-spiked and fermented extrusion products caused moderately severe kidney lesions and reduced kidney weights, effects typically found in fumonisin-exposed rats. Lesions in rats fed contaminated grits after extrusion with glucose were significantly less severe and not accompanied by kidney weight changes. The authors concluded that extrusion with glucose supplementation is potentially useful for safely reducing the toxicity of fumonisins in maize-based products. Lu et al (2002) had reached the same conclusion and shown that glucose bind to fumonisins via the amino group. Dall´Asta et al. (2010), however, have found a higher amount of total detectable fumonisins in *in vitro* digested food in comparison with the nondigested matrix, an amount even higher than that calculated through the application of the hydrolysis procedure.

61. Jackson et al. (2012) summarized studies published about the fate of FB1 in maize submitted to extrusion under different conditions. The review indicated that FB1 stability during extrusion depends on the temperature, screw speed and presence of reducing sugars. They also showed that *in vivo* bioassays conducted so far indicated that extrusion, especially with glucose, is a useful tool to reduce FB1 toxicity of contaminated maize.

62. Voss et al. (2011) tested whether extrusion, with or without glucose supplementation (10%), is an efficient technique in reducing FB1 toxicity. They fed male rats with naturally contaminated extrused maize grits and found out that extrusion with glucose significantly reduced FB1 intakes and prevented the development of kidney lesions and disruption of sphingolipid metabolism.

63. Nixtamalization can reduce fumonisin concentration from 50 to 80%, with 35 to 60% of fumonisin being detected in its hydrolyzed form (Burns et al., 2008; Dombrink-Kurtzman et al., 2000). 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 FB1, however, the masa product exhibited about 60% of the toxicity of the untreated maize using a brine shrimp assay procedure (Park et al., 1996). Burns et al. (2008) suggested that mycotoxin-in-corn matrix interactions during nixtamalization reduce the bioavailability and toxicity of FB1 in rats.

64. Palencia et al (2003) found that tortillas prepared using the traditional nixtamalization method of Mayan communities contained FB1, FB2 and FB3 and their hydrolyzed counterparts. There were equimolar amounts of FB1 and HFB1 in the tortillas, but the total fumonisins were reduced by 50%. They also found a reduced sphinganine elevation in cells treated with extracts of tortillas compared with cells treated with extracts of contaminated maize.

65. Voss et al. (2013) showed with *in vivo* experiments that nixtamalization is effective in the reduction of the potential toxicity of corn contaminated with FB1. They fed male rats, during 3 weeks, with diets containing low, medium or high levels of FB1-contaminated raw corn or alkaline cooked corn. Significantly increased sphinganine and sphingosine concentrations in the kidneys and FB1 characteristic kidney lesions were found in animals fed with uncooked for all levels tested, while in the alkaline cooked contaminated with the highest level the effects were less intense.

66. Ethanol fermentation of fumonisin contaminated maize 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 (Bennett and Richard, 1996; Bothast et al., 1992). Fumonisins have also been found in beer, indicating that the toxins persist under the conditions (temperature, pH) prevailing during the brewing process (Scott & Lawrence, 1995; Hlywka & Bullerman, 1999).

67. Visconti et al. (1996) found that gamma-irradiation (15 kGy) effectively sterilized the maize flour, but caused only about a 20% reduction in its fumonisin content. Ferreira-Castro et al (2007) found possible decreased fumonisins levels by irradiating maize with 5 or 10 kGy; however, at 2 kGy, the survived fungi (36%) were able to produce more fumonisins than the fungi in the control samples. Aziz et al. (2007) found that the viable counts of *Fusarium* in seeds decreased by increasing the radiation dose levels; 7 kGy was sufficient for complete destruction of FB1 in wheat and maize.

68. In a study by D'Ovidio et al. (2007), fumonisins in naturally contaminated whole kernel and ground corn samples irradiated by both gamma and electron beam at increasing levels were not reduced significantly. However, *Fusarium* spp. was totally eliminated at 30kGray in the ground corn and at 100 kGy in whole corn. In the same study, popcorn kernels rejected from the cleaning process were popped in a microwaved, resulting in significant reduction of fumonisins.

69. Mycotoxins can be degraded or converted into less toxic molecules by biological decontamination with enzymes and selected microorganisms. More recently, biological decontamination and biodegradation of mycotoxins with microorganisms or enzymes have been used (He et al, 2010). Many species of bacteria and fungi have been shown to enzymatically degrade mycotoxins (Bata & Lasztity, 1999; Pereira et al, 2010. Black yeast strains (Duvick et al., 1998) and bacterial strains (Benedetti et al., 2006; Heinl et al., 2010) can catabolize fumonisins. However, question remains on the toxicity of products of enzymatic degradation and undesired effects of fermentation with non-native microorganisms on the quality of food (Shetty & Jespersen, 2006).

Zearalenone

70. During cleaning and sorting 84% of zearalenone could be removed (EFSA, 2011).Palpacelli et al. (2007) have shown that stone milling results in a 40-50% reduction of zearalenone in wheat flour, which was significantly lower compared to use of a modern roller mill. Wolff (2005) studied the effect of sorting, cleaning and milling on the zearalenone concentration. The by-products from cleaning the raw cereal grains (dust, hulls and others) contained 3- to 30-fold higher zearalenone concentrations than the cleaned cereal grains, while concentration in bran was up to 2-fold higher than in grain.

71. Ryu et al. (2002) showed that during the dry milling of corn, wheat, barley, and other cereals, zearalenone was found in highest amounts in fractions of the commodity that are less likely to be used for food production (germ and bran fractions). In the wet milling of corn, mycotoxins, including aflatoxin, zearalenone and fumonisins, can be found in the steep water, gluten, fiber and germ, while the starch tends to be relatively free of these mycotoxins (Lauren and Ringrose, 1997; Ryu et al., 2002. Several studies reported no detectable zearalenone in starch from wet milling (Bennett et al., 1978; Romer, 1984; Bennett et al., 1978b).

72. A wide variety of chemicals, including calcium hydroxide monomethylamine, sodium bisulfite, moist and dry ozone, chlorine gas, hydrogen peroxide, ascorbic acid, hydrochloric acid, sulfur dioxide, formaldehyde, ammonia and ammonium hydroxide, have been found to be effective (at different extents) against several Fusarium mycotoxins, including zearalenone, DON, T-2 toxin, and fumonisins (Visconti, 2001).

73. Heating of pure zearalenone or zearalenone contaminated grain for several hours at 150°C did not lead to a significant loss of zearalenone. Starting at 200°C a moderate degradation was observed (Lauren and Smith, 2001). Heating of zearalenone in an aqueous solution or under alkaline conditions led to degradation starting at 150°C whereas boiling in water did not influence the zearalenone content (Ryu et al., 1999).

74. According to EFSA (2011), only under alkaline conditions or during extrusion cooking (heating under a high degree of pressure) a reduction of above 40% was observed. Extrusion cooking can reduced zearalenone levels in food as well as its estrogenic activity (Ryu et al., 2002).

75. A high degradation rate was observed during extrusion of maize grits or maize meal spiked with zearalenone. Depending on the extrusion parameters (temperature, moisture content, screw speed) the zearalenone concentration was reduced by 66-83% (Ryu et al., 1999), 66-81% (Cetin and Bullerman, 2005) and 6-54% (Scudamore et al., 2008a). The loss of zearalenone seems not to be affected much by the presence of dextrose. The addition of salt resulted in higher zearalenone levels indicating a lower reduction rate (Scudamore et al., 2008a). Extrusion cooking of wheat spiked with 305 µg/kg of zearalenone resulted in a reduction rate of 3-17% depending on the parameters used (Scudamore et al., 2008b).

76. During the traditional preparation of tortillas using naturally contaminated corn the reduction ranged from 59 to 100% (Abbas et al., 1988). This strong degradation can be explained by the alkaline conditions during nixtamalization.

77. In bread-baking experiments at approximately 200°C for 30 minutes using wheat flour with a zearalenone concentration of 1-20 mg/kg, 34-40% of zearalenone was degraded. The production of instant noodles with the addition of 1% potassium carbonate resulted in a 48-62% reduction of zearalenone. In biscuits with 3% sodium bicarbonate zearalenone was decreased by 16-27% (Matsuura et al., 1981).

78. Fermentation of foods with bacteria and yeast resulted in reduction in zearalenone levels, however, this process can result in the conversion of zearalenone to more potent derivatives such as α-zearalenol (Ryu et al., 2002).

79. Zearalenone also may be transferred from contaminated grains into beer, in the brewing process (Bullerman & Bianchini, 2010; Scott, 1996), while no mycotoxins have been detected in ethanol produced from contaminated cereals (Visconti, 2001). The source of these mycotoxins could be the malted grain or adjuncts, such as corn in the form of grits or syrup, rice grits, unmalted barley, wheat starch, or sorghum grits, which are used to provide fermentable carbohydrates for the yeast (Hoseney, 1994; Scott, 1996). Kocić-Tanackov (2007) showed that during micro-malting zearalenone content increased. Zearalenone content determined in finished malt was higher than in barley.

Deoxynivalenol (DON)

80. There is a close relationship between the percentage of *Fusarium*-damage kernels and DON content (Beyer et al., 2007). Based on the analysis of Fisher-transformed r values (zr values), *Fusarium* damage kernels had the strongest relationship with DON, with a mean r of 0.73 (Paul et al., 2005).

81. Debranning of wheat, a mechanical process by which the outer layers of wheat grains are removed prior to the milling process, is used in industrial processing as it can enhance the milling performance of wheat and the degree of refinement of flour and semolina (Dexter & Wood, 1996). However the effect of debranning and the efficiency of mycotoxin removal are variable (Cheli et al., 2013).

82. In a study of the fate of mycotoxins in wet milling for starch production investigators found either nil or trace (close to detection limits) of DON in starch (Lauren et al., 1997).

83. Milling reduces mycotoxin concentrations in fractions used for human consumption, but concentrates mycotoxins into fractions commonly used as animal feed. However, these fractions may represent promising novel food ingredients with a high value for human nutrition, too, as with bran (Cheli et al., 2013). During milling the highest concentrations of DON were found in the bran, the lowest in the reduction flour (Lancova et al., 2008).

84. Extrusion cooking of corn flour is effective (higher than 95%) for the inactivation of DON in all tested conditions: 150°C and 180°C, 15 and 30% flour moisture, with or without sodium metabisulphite addition (1%) (Cazzaniga et al., 2001).

85. Treatment of contaminated maize (4.4 mg/kg) with sodium bisulfite solutions at 80°C for 18h can reduce about 85% of DON into a its sulfonate conjugate, which appears to be nontoxic to pigs (Young et al, 1987). Other chemical treatment with hydrochloric acid, hydrogen peroxide, sodium hypochlorite, ascorbic acid and ammonium carbonate, did not prove any efficiency against DON (Jouany, 2007).

86. Bakery processing has been reported to reduce DON contamination by some authors (Abbas et al. 1985, Seitz et al. 1986, Boyacioglu et al. 1993), while others suggested that DON is highly stable in this process (El-Banna et al.1983, Scott et al. 1983, 1984). Moreover, DON appeared to be very stable at 100°C at the pH of the bread (Wolf and Bullerman 1998). Baking at 210 °C for 14 min had no significant effect on DON levels according to Lancova et al. (2008). Pacin et al (2010) suggested that any reduction is a result of not only from thermal decomposition but also fermentation losses.

87. A new approach for reducing the absorption of DON from contaminated food was reported by Tamura et al. (2013). The method uses low-methoxyl pectin gel to trap the DON physically, and significantly suppressed its absorption in the gastrointestinal tract. The suppression would not affect the absorption of essential nutrients as the gel degraded in intestine. Further studies are required to formulate applications for cereal or corn-based foods contaminated with DON.

Ochratoxin A (OTA)

88. Sorting and cleaning are not important to reduce OTA content, as only 2-3% reduction of OTA in barley was achieved by cleaning (Scudamore et al., 2003).

89. Removal of the surface layers by abrasive scouring or polishing and milling to remove outer layers for white flour production lowers OTA levels, since the mycotoxin tends to be concentrated in the outer bran layers of cereals (Duarte et al, 2010).

90. OTA is a moderately heat stable molecule that can survive most food processing operations and, therefore, it appears in final and derived products (Bullerman and Bianchini, 2007). However, under certain conditions of high temperature, acidic or alkaline conditions or in the presence of enzymes breakdown can occur (Scudamore, 2005).

91. During the white bread manufacturing process, grain cleaning by scouring the removal of the bran and offal fractions decreases OTA content by up to 25%. Heat treatment during flour and bread production does not seem to affect OTA levels (Scudamore et al., 2003).

92. The stability of OTA during extrusion of contaminated whole meal wheat flour was examined using pilot scale equipment. Factors examined were temperature, moisture content, screw speed and residence time. OTA was partially stable, with breakdown increasing with temperature and moisture content. However, even under the harshest conditions likely to be used in commercial practice, maximum loss was no greater than 40%, with a residence time of about 40 seconds. The chemical properties of OTA suggest that breakdown might be affected by changes in pH and that further studies are necessary to investigate this possibility (Scudamore, 2004).

93. According to Duarte et al. (2011) autoclaving oatmeal with 50% water resulted in a loss of 74% of the OTA content, while applying the same process to dry oatmeal or rice cereal lead to greater reductions (86 e 87.5%) (Bullerman and Bianchini, 2007). The extrusion processing, largely used in breakfast cereal production, can also reduce the levels of OTA. Scudamore et al. (2004) studied the stability of OTA during extrusion of contaminated whole meal wheat flour, observing that a higher temperature and moisture content lead to a bigger OTA breakdown. Degradation was also increased by longer residence time, when lower mass flow rates were applied, because the time the product spent in the extruder was increased. However, the maximum loss observed was no greater than 40% of the initial amount of OTA (Bullerman and Bianchini, 2007).

HACCP

94. The hazard analysis and critical control point (HACCP) has become awidely recognized and recommended method for food safety assurance (WHO, 2007). This tool is of great importance to define which steps of the processing should be controlled to avoid the presence of mycotoxins in ready-to-eat products.

95. The HACCP principles are a way of operating and depend on the technical and economical capability of the implementing countries (Pitt et al., 2012). Some control measures may depend on investments in equipment and analytical control.

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APPENDIX IV

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