



FEED THE FUTURE

The U.S. Government's Global Hunger & Food Security Initiative



USAID/MAIL/KSU/FTF PHL-IL “Rapid Assessment of Mycotoxins in Afghanistan’s Food Value Chains”



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1. Summary

The Feed the Future Innovation Lab for the Reduction of Post-Harvest Loss undertook a base-line study of mycotoxin contamination in nuts (pistachios, almonds and walnuts), raisins and wheat in Afghanistan. A laboratory for performing ELISA analyses was established in Kabul under the auspices of MAIL, and staffed by 12 MAIL employees supervised by Ms. Debra Frey. Samples were collected from local markets and analyzed by staff at the MAIL laboratory and selected samples also were evaluated at ISPA, BOKU, the University of Nebraska and Kansas State University. Assays conducted in Nebraska and Kansas used the same technology used in Afghanistan. Assays done at ISPA and BOKU used state-of-the-art analytical chemical techniques to both validate the results obtained in Kabul and to determine if toxins other than those detectable with the commercially available ELISA kits were present.

Establishing an analytical laboratory and training a technical support team was an important capacity building effort. The laboratory in Kabul was functional and technicians were capable of running mycotoxin assays on a routine basis. The staff should be capable of running similar assays for these and other foodborne contaminants if supplies and samples are available to do so. Additional training is needed to reinforce initial lessons on sampling strategies, to strengthen quality control of results obtained, and to improve record keeping and data management of the results. Results obtained during this study were for research, information and training purposes, and were neither collected nor analyzed in a manner that would satisfy more rigorous regulatory enforcement requirements. The lab and its equipment were disassembled and stored at the end of the project, and should be available to for the World Bank sponsored MAIL laboratory for mycotoxin analyses that is targeted for the same space occupied by the lab established for this project.

Mycotoxin assays frequently are problematic because of the variation within the sample. A single contaminated nut, raisin or wheat kernel may suffice to push an entire sample above a critical threshold. ELISA assays also are subject to experimental error that can result if kits have been shipped or stored improperly in addition to any errors associated with operator error. These assays have a history of reporting higher levels of contamination than found in more sophisticated chemical tests, and that pattern held for this study as well. In this report we provide the data collected at BOKU in Austria, which used a LC/MS/MS protocol to simultaneously detect 600 + metabolites at one time. Interpreting the results in terms of whether they are above/below critical cut-offs is the most resilient way to understand these data. Nuts and raisins were screened in Afghanistan for the presence of aflatoxins and ochratoxin A, while wheat was screened for aflatoxins, ochratoxin A, and the trichothecenes deoxynivalenol, HT-2 and T-2.

For nuts, the analyses from Afghanistan suggested that nearly half of the almond and walnut samples, had problems with aflatoxin contamination, but these results were not supported by the multi-mycotoxin assays which found no aflatoxins in the walnuts and only 6/89 almond samples with levels that would lead to rejection by the EU. More than 50% (26/46) of the pistachio samples were detectably contaminated with aflatoxins, some as high as nearly 3000 µg/kg, and 26% at levels that would lead to rejection by the EU. *Aspergillus* metabolites were found in 43/46 samples, suggesting that the potential for aflatoxin contamination to increase with storage time is good. Ochratoxin A contamination was detected only at low levels in two walnut (<0.8 µg/kg) and two pistachio (2.5 µg/kg) samples, and in none of the almond samples.

In raisins, the results obtained in Afghanistan and at BOKU differed for aflatoxins, with the Afghanistan tests finding nearly 50% of the samples contaminated with aflatoxin while none were positive in the BOKU analyses. The reasons for this discrepancy are not known, but could include

the test kit, technical errors in extraction and assay, and non-random splitting of the samples of the samples that were analyzed. Nearly 10% of the samples examined had ochratoxin A levels that would limit exports to the EU, and an additional 14% had some level of contamination. With 99% of the samples carrying evidence for contamination by *Aspergillus niger*, the likelihood of major ochratoxin contamination problems on exported raisins is high.

Wheat is the major staple cereal in Afghanistan with an average Afghan reported to consume ~500 g of wheat daily. Contamination thresholds for food safety estimates are based on common Western diets, and contain much less wheat than is commonly consumed by an average citizen of Afghanistan. The large amount of wheat consumed by Afghans means that even levels of contamination that would be considered “safe” in a Western context may be problematic in an Afghan diet. Thus the frequency at which contaminated samples were detected is probably at least as important a risk factor as the level of contamination present in the samples. Based on the BOKU data for 153 samples, 4% of the samples were positive at some level for aflatoxins, 12% were positive for ochratoxin, 2% were positive for T-2/HT-2, 2% were positive for zearalenone, and 33% were positive for ergot alkaloids. Results from ELISA tests used in Afghanistan to detect T-2 were not consistent and when samples were tested with more sophisticated chemical techniques, T-2/HT-2 was detected at low levels in only four of the 153 samples. Thus, neither zearalenone nor T-2 appears to pose major public health risks in wheat in Afghanistan. Aflatoxin contamination was unexpected, as it is not an issue on commercial wheat produced in Western countries. Poor storage conditions, however, could lead to post-harvest contamination. Ochratoxin A can be a problem in wheat in northern Europe and animals that consume contaminated grain may accumulate the toxin in their muscle. The frequency of ochratoxin A contamination is high enough to be of concern for public safety in Afghanistan, especially since this toxin is associated with kidney failure and this medical condition is a known health problem in the country. Of the 19 contaminated samples, 6 exceeded the European maximum for ochratoxins in cereals for human consumption. Ergot alkaloids were identified in wheat only through the multi-metabolite analyses conducted at BOKU. This class of compounds was not a target of the original test protocol, and the few ELISA tests available for these compounds are focused on regulations for animal feeds rather than human food. Ergots were detected in 50/153 samples suggesting that an ergot epidemic had occurred during the crop year from which samples were taken. Ergot is a disease that occurs periodically (depends upon environmental conditions) on grasses and small grains and can be problematic in countries where grain milling options are limited.

The project provided a training session on mycotoxin detection for MAIL staff on 28-29 July 2015 that focused on the technology and analytical procedures (Appendix III). It also sponsored a conference in New Delhi (14-16 March 2016) at which results from the project were shared with stakeholders and nominal group discussions were held to identify paths forward. A follow-up to this meeting was held at the US Embassy in Kabul on 16 July 2016 to discuss communication strategies.

Potential follow-up activities are numerous. Those that seemed of highest priority include:

- Continued evaluations of background mycotoxin contamination to increase the capacity of the lab in Afghanistan and to generate data on important variables such as cropping and storage conditions and environmental factors.
- Development of management and communication strategies on mycotoxins to which all three ministries agree, and increasing the human capacity within the ministries to enable the delivery of information to multiple audiences and to address at least basic questions in country.

- Design short, medium and long-term training programs to increase human capacity in country to detect and provide remediation plans for mycotoxin contamination.

2. Project design

2.1 History and implementation. The original Scope of Work is in Appendix I and the Grant Document Modification is in Appendix II. The partnership structure and operational design of the project was constructed with elements of both capacity building and national engagement, as well as delivery of sound technical results. Given the original remit to conduct a survey of which toxins are present in which commodities, and the end of project request to develop a risk communications strategy, delivery on both sides required balancing. In order to fulfil the national engagement, sensitization and capacity building at a national level, discussions with both the Ministry of Agriculture, Irrigation and Livestock (MAIL), as well as the Ministry of Health were held early during the project period. Preliminary evaluations of the capacity of MAIL to support the project were made as part of the project's design (Appendix VI). Furthermore, staff from MAIL were trained and enlisted to conduct sample collection and sample analysis in a newly established lab, through the project (see Appendix III for training materials, and Appendices IV and V for protocols used in the lab in Kabul). However, establishment of robust, proficient laboratory analysis for mycotoxins is not a trivial matter. To help ensure delivery of reliable, robust results, international partners were included, who received samples from MAIL and conducted their own high-end, sophisticated multi-mycotoxin analysis. Through this design, the risk associated with relying on high-quality results from a recently established lab was mitigated by inclusion of world-class, established operations; and the involvement of the national partners was not compromised, given the involvement of MAIL in discussions and hands-on involvement leading collections and conducting analysis. In the end, both the national engagement/capacity building and the robust survey of mycotoxins in the target commodities were achieved.

The project was initiated following e-mail and phone discussions between USAID Afghanistan Ag Officer McDonald Homer and John Leslie, University Distinguished Professor of Plant Pathology at Kansas State University in Manhattan, Kansas. A buy-in to the Feed the Future Innovation Lab for the Reduction of Post-Harvest Loss was used to fund the effort (Appendix II), with Prof. Dirk Maier as the initial P.I. When Prof. Maier left K-State in August 2015, Prof. Leslie became the P.I. for the project.

The project's in-country component included Ms. Debra Frey as the Project Coordinator, and a staff of MAIL employees who were selected and trained by Ms. Frey (Figure 1). Upon arrival in Afghanistan in July 2015, Ms. Frey had to completely set up a lab in two empty rooms, including adding much of the essential infrastructure – air conditioning, back-up power, *etc.* She also provided initial training (Appendix III) for the staff as a group and continuing training for staff on an ongoing basis as the project progressed. The equipment used in the lab and to make the lab operational have been disassembled and are to be incorporated into the mycotoxin analysis lab being established by MAIL with World Bank funds, probably at the same location at which the USAID/KSU lab was established.

Figure 1. K-State/MAIL team assembled for this project.



Samples were collected and placed into one of several classes depending on the variety of the material screened, and location where the sample was collected (Tables 1-5). Data were analyzed for this report based solely on the crop material from which they were collected. In a number of cases, sample numbers were too small for underlying patterns to be clearly discerned, so this additional detail was not included in the analysis. Samples were evaluated in Afghanistan, Kansas State University and University of Nebraska-Lincoln with an appropriate, quantitative Romer Labs diagnostic kit (ELISA based). At the Institute for Science of Food Production (ISPA) in Bari, Italy, several analytical tests were used to evaluate wheat for the presence of one or more trichothecene mycotoxins (T-2, HT-2, neosolaniol, diacetoxyscirpenol, deoxynivalenol, and nivalenol). At the Austrian Agricultural University (BOKU) in Tulln, Austria, an LC/MS/MS multi-mycotoxin assay was run which is capable of detecting up to 650 small molecule metabolites at one time was run. Of the 627 samples collected, 606 were evaluated with this multi-mycotoxin methodology (Appendix VII). Regulated mycotoxins are reported separately (Appendix VIII) and the presence of others that indicate the presence of particular fungal genera are used to infer the presence of more problematic fungi in the samples analyzed (Appendix IX). No mycological analyses as such were included in the study.

Table 1. Wheat flour and grain samples.

	Type of Sample	Total Number of Samples	Samples Analyzed in Austria
W01	Asiabs mill flour of Afghan origin (grain often stored on dirt floor next to a stream)	71	66
W02	Grist mill flour of Afghan origin (usually stored on a cement floor and in a cement structure)	88	87
W03	Asiabs and grist mill flour of Kazakhstan origin	4	4
W04	Asiabs and grist mill flour of Uzbekistan origin	1	1

W05	Purdue Improved Crop Storage (PICS) bags – stored grain	1	1
W06	Two warehouses or other storage facilities in each region, as recommended by grain traders or farmers.	11	9
W07	Two naan bakeries in each region (Either dirt or cement floors, see sample IDs)	26	25
W08	Two-four commercial flour millers in each region	3	3
W09	Pakistan flour	12	11
W11	Other flour	2	2
W12	Turkmenistan flour or wheat	3	3
	TOTAL	222	212

Table 2. Almond samples.

	Type of Sample	Total Number of Samples	Samples Analyzed in Austria
A01	Sattarbai soft-shell almonds	23	23
A02	Shokorbai hard-shell almonds	6	6
A03	Abdul Wahidi almonds	15	15
A04	Qambari almonds (very strong almond flavor)	15	15
A05	Ghorbandi almonds	2	2
A06	Sangaki and Murawaji almonds (smaller kernels)	21	21
A07	Other almonds	14	12
	TOTAL	96	94

Table 3. Raisin samples.

	Type of Sample	Total Number of Samples	Samples Analyzed in Austria
R01	Medium quality round green raisin (dried in the shade and in mud houses-Kishmish Khana)	29	28
R02	Medium quality long green seedless raisin (dried in the shade and in mud houses-Kishmish Khana)	36	35
R03	High quality Shundurkhani raisin (Golden – High value, dried in the shade and in mud houses-Kishmish Khana)	24	24
R04	Medium quality red raisin (sun dried locally and used in rice dishes and baked goods)	33	33
R05	Sun-dried Shomali raisin (sun dried, black in color, has a strong concord grape flavor but	26	26

	Type of Sample	Total Number of Samples	Samples Analyzed in Austria
	small seeds, often exported to the former Soviet Union to make cognac (Kvass)-like product]		
R06	Sun-dried Ghazni raisin [sun dried, black in color, has a strong concord grape flavor but small seeds, often exported to the former Soviet Union to make a cognac (Kvass)-like product]	19	19
R07	Sun-dried Tayefee (northern Afghanistan variety name) & Abjous (southern Afghanistan variety name) raisins (dipped in sulfur and sun dried on the dirt, has a dried fig flavor)	14	14
R08	Small red raisin or currant (sun dried and stirred in dirt, locally used raisins in rice dishes and baked goods)	18	18
R09	Other or mixed raisins	4	4
	TOTAL	203	201

Table 4. Pistachio samples.

	Type of Sample	Total Number of Samples	Samples Analyzed in Austria
P01	Korak pistachios (open shell with purple outer skin)	22	18
P02	Pushdara pistachios (closed shell with purple outer skin)	12	11
P03	Khandan-e-safid pistachios (strong flavor and wrinkly shell)	11	9
P04	Other varieties of pistachios	10	10
	TOTAL	55	48

Table 5. Walnut Samples.

	Type of Sample	Total Number of Samples	Samples analyzed in Austria
WN01	Zard walnuts (yellow kernels)	9	9
WN02	Mazaari walnuts (variety from Mazar with unique flavor)	3	3
WN03	Takhari walnuts (variety from Takhar province with unique flavor)	2	2
WN04	Korak walnuts (opening in shell)	7	7
WN05	Kaghazi walnuts (paper shells)	13	13
WN06	Other varieties of walnuts	17	17

	TOTAL	51	51
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2.2 Deliverables identified in the Scope of Work (Appendix I)

2.2.1 Detailed Methodology and Action Plan

The PHL Innovation Lab will develop a detailed work plan and a brief suggested methodology to be reviewed and approved by project management. This should be done no later than the end of first month of mobilization.

The plan of work was developed and modified several times to reflect realities associated with getting materials and equipment to Kabul, waiting until after Ramadan to begin the study, and the time required for hands-on training in sample collecting and processing. The June 15th time line is attached as Appendix X. This time line was amended later in the project, but it encompasses all of the projected activities. Protocols used are described in Appendices IV and V, in presentations made as part of the final debriefing for USAID in December 2015 (Appendix XI) and as part of the presentations made at the Delhi workshop (Appendix XII.6, XII.8 and XII.9).

2.2.2 Progress Updates

The PHL Innovation Lab will compile a brief progress update on a bi-weekly basis using a report template agreed to by sponsor and awardee prior to project start.

Written progress reports were provided in June (Appendix IV.4) and in August 2015 (Appendix IV.5). Weekly phone/video conferences were held from May 2015 through February 2016 that routinely included staff from USAID-Kabul and PHL in Kabul, Lincoln and Manhattan, and as needed included staff from USAID-Washington, ISPA (Italy) and BOKU (Austria). These weekly meetings were used to discuss results and current and coming activities associated with the project.

2.2.3 Final Report

The outline of the report will be developed in the inception phase. Both hard and soft copies of the reports prepared in MS-Word will be provided along with relevant literature reviewed.

This document.

2.2.4 Stakeholder Briefing

A final briefing will be held by the PHL Innovation Lab for MAIL. USAID representative/s and key stakeholders will be present to reflect on the major findings and recommendations.

An all-day debriefing was provided for USAID-Kabul and other local stakeholders by Ms. Frey and staff at the KSU/MAIL lab. Materials used in this presentation are in Appendix XI.

2.2.5 MAIL Lab support

In addition to providing training to MAIL staff, this project provides support for the equipping of MAIL labs to help with the continuation of mycotoxin research and detection. The PHL Innovation Lab will identify, select and order the equipment to be purchased for conducting the mycotoxin analysis of the field samples collected. MAIL staff will be trained on the use of this equipment, and that equipment will remain in the MAIL labs after this project is concluded.

The lab was equipped with ELISA readers and equipment necessary to prepare and analyze samples was purchased. MAIL staff were instructed in the use and protocols to be followed. All equipment shipped to Afghanistan was left in the lab when the on-the-ground portion of the project ended in December 2015. This lab has been closed and the equipment provided has been stored for use in a WHO/MAIL lab to be established for doing mycotoxin analyses.

2.2.6 International Workshop

USAID will circulate the results of this investigation among development partners with the intent of prompting further research and appropriate measures to improve food quality and reduce post-harvest losses. The Awardee will organize an international workshop on post-harvest losses and food quality towards the end of this project where the results and mitigation recommendations will be presented. This workshop will take place outside of Afghanistan in the summer of 2015.

This workshop was held in New Delhi in March 2016. Materials used in the presentations and the agenda are in Appendix XII. Dr. Leslie traveled to Kabul after this meeting to discuss the general results reported at the Delhi meeting with USAID and other stakeholders in Kabul.

3. Samples analyzed and results

3.1 General Comments. Samples were all collected by the MAIL team in Kabul from various locations in Afghanistan. Most samples were taken from markets, mills and other local aggregation points. These samples are certainly representative of what is being consumed locally, but may not be representative of what is grown locally, as some samples were taken of wheat sourced from outside Afghanistan when identified as such by the seller. Data on location, variety and growing/processing conditions also are available, but have been excluded from this report to help simplify the case being made. Fungi inferred to be present are based on the presence of one or more metabolites known to be produced by those fungi in the multi-mycotoxin screen. European regulations are used as the maximum allowable levels for exports (nuts and raisins). The European diet is quite different from the Afghan diet. The differences in the amount of wheat consumed per day, result in Afghans being exposed to much higher total amounts of toxins in wheat than are Europeans, even though the contamination levels might be lower overall in the food being consumed. Thus data for wheat contamination are not discussed in terms of contamination relative to European standards.

Data were analyzed in Afghanistan, BOKU (Austria), ISPA (Italy), University of Nebraska-Lincoln, and Kansas State University. No two locations evaluated exactly the same set of samples, although there were numerous overlaps between the sets of samples analyzed. In general, samples analyzed outside Afghanistan were more consistent with one another than they were with the results collected in Afghanistan. In general, results from Afghanistan report higher levels of toxins present than do the other methodologies. In at least one case, the tests being used in Afghanistan for T-2 and HT-2 were confirmed to be faulty after follow-up tests were conducted with both more sophisticated chemical methods at ISPA and BOKU and with replica ELISA tests from multiple manufacturers in both Kansas and Nebraska.

3.2 Key findings. More detailed analyses for each commodity follow in the section below, as well as comments on the methodology used and its reliability.

Raisins – **Ochratoxin** contamination of raisins is problematic as 10-14% of the samples evaluated contained ochratoxins at a level precluding export to the European Union (Table 6). **Aflatoxin** is probably not a problem for raisins, although the results were dichotomous. None of the samples evaluated by LC MS/MS in Austria had levels that would prevent export to the EU, but 46% of the samples evaluated by ELISA in Afghanistan were reported with levels that would prevent export to the EU.

Almonds – **Ochratoxin** was not detected as a problem in the almond samples assayed by any method at any location. **Aflatoxin** contamination of almonds is problematic as 7% of the almonds assayed for aflatoxin had levels above those allowed in the EU when assayed by LC MS/MS. ELISA assays were more variable with 42% of almonds assayed over the limit based on ELISA assays conducted in Afghanistan and either 5 or 16% of samples over the limit depending on the ELISA kit used when tested at the University of Nebraska-Lincoln. Outside Afghanistan, aflatoxin is not usually a major contaminant of raisins.

Pistachios – **Ochratoxin** was not detected as a problem in the pistachio samples assayed by any method at any location. **Aflatoxin** is a major problem in pistachios, as 30% of the pistachios assayed for aflatoxin had levels above those allowed in the EU when assayed by LC MS/MS. ELISA assays were more variable with 68% of pistachios assayed over the limit based on ELISA assays conducted in Afghanistan and 17-18% of samples over the limit when assayed with ELISA kits at the University of Nebraska-Lincoln.

Walnuts – **Ochratoxin** was not detected as a problem in the walnut samples assayed by any method at any location. **Aflatoxin** is unlikely to be a problem in Afghanistan walnuts. None of the walnuts assayed for aflatoxin had levels above those allowed in the EU when assayed by LC MS/MS or the Neogen ELISA test kit. In Romer ELISA assays 50% of walnuts were over the EU limit when assayed in Afghanistan and 35% were over the limit when assayed at the University of Nebraska-Lincoln.

Wheat – **Ergot alkaloids** were a problem in wheat and were found in one third of the samples assayed. The *Fusarium* trichothecene toxins, **deoxynivalenol** and **T-2 toxin**, were not important contamination problems and were present in only a few samples. **Ochratoxin** contamination is a minor problem as 1-5% of the samples are contaminated at levels above the EU threshold. **Aflatoxin** contamination may be a problem in wheat as this toxin was detected in ELISA assays in Afghanistan and the United States, but not in the LC MS/MS assays. The significance of the ochratoxin and aflatoxin contamination that is occurring may be underestimated by using EU thresholds to determine if significant contamination has occurred because the Afghan diet contains much more wheat (~500 g/day) than does the European diet that was used to develop the EU guidelines.

3.3 Raisins. ELISA tests run in Afghanistan detected aflatoxin in numerous raisin samples (Table 6). In contrast, none of the samples evaluated at BOKU had detectable aflatoxins. In general, the major problem with raisins being imported into Europe is ochratoxin contamination rather than aflatoxin contamination. The results from BOKU are consistent with this broad general pattern, and we conclude on this basis that aflatoxins probably are not a major contamination problem on raisins from Afghanistan even though we have not identified a specific reason for the large number of false positives detected by the in-country lab. Ochratoxin is a significant problem, with 47/204 samples containing detectable ochratoxin and with 18 samples contaminated at

Table 6. Comparison of aflatoxin and ochratoxin assays as samples leading to rejection at European Union levels or US/International levels.

Analysis	Aflatoxin				Ochratoxin			
	# of samples ^a	<EU ^b	>EU, <US ^c	>US ^d	# of samples	<LOD ^e	<EU	>EU ^f
Raisins								
LC/MS/MS ^g	198	100	0	0	197	85	5	10
ELISA – AFG ^h	102	54	43	3	104	71	15	14
Almonds								
LC/MS/MS	89	93	3	4	87	100	0	0
ELISA – AFG	72	58	34	8	72	100	0	0
ELISA – R/UNL ⁱ	82	84	12	4	82	100	0	0
ELISA – N/UNL ^j	82	95	1	4	82	100	0	0
Pistachios								
LC/MS/MS	46	70	15	15	47	96	4	0
ELISA – AFG	28	32	28	40	43	100	0	0
ELISA – R/UNL	39	62	17	21	39	100	0	0
ELISA – N/UNL	39	59	18	23	39	100	0	0
Walnuts								
LC/MS/MS	27	100	0	0	26	100	0	0
ELISA – AFG	36	50	25	25	36	100	0	0
ELISA – R/UNL	26	65	35	0	26	100	0	0
ELISA – N/UNL	26	100	0	0	26	100	0	0
Wheat								
LC/MS/MS	156	100	0	0	156	96	3	1
ELISA – AFG	126	65	32	3	113	76	19	5
ELISA – R/UNL	185	81	19	0	185	92	8	0
ELISA – N/UNL	186	100	0	0	80	100	100	100
ELISA – R/KSU ^k	217	100	0	0	219	98	1	1
ELISA – V/KSU ^l	-	-	-	-	219	26	73	1

^aNumber of samples analyzed.

^b% of samples with toxin levels less than the EU threshold for rejection.

^c% of samples with toxin levels between the EU and US thresholds for rejection.

^d% of samples with toxin levels exceeding the US threshold for rejection.

^e% of samples with toxin levels below the level of detection for the ELISA assay.

^f% of samples with toxin levels exceeding the EU threshold for rejection.

^gAssay conducted at BOKU in Tulln, Austria.

^hAssay conducted in Afghanistan at MAIL/KSU laboratory with a Romer ELISA test kit.

ⁱAssay conducted at University of Nebraska-Lincoln with a Romer ELISA test kit.

^jAssay conducted at University of Nebraska-Lincoln with a Neogen ELISA test kit.

^kAssay conducted at Kansas State University with a Romer ELISA test kit.

^lAssay conducted at Kansas State University with a Viacam ELISA test kit.

a level above 8.0 µg/kg (ppb) – the EU maximum allowable contamination. Fumonisin B₂ also was detected in ten samples at levels ranging from 5.4-25.7 µg/kg. These levels are at best 4% of regulated values in maize and probably do not pose a health risk and are not regulated at such low levels. Although usually considered a *Fusarium* metabolite, in this case the FB₂ probably was produced by one or more strains of *Aspergillus niger*, as this species is known to be able to synthesize low levels of this toxin.

In terms of fungi, all but two samples contained metabolites produced by *Aspergillus niger*, with the two species lacking *A. niger* related metabolites not containing any of the other metabolites associated with *A. niger* either. Nearly 70% of the samples (141/204) contained metabolites associated with species of *Aspergillus* other than *A. niger*, indicating that most of the raisins were colonized by multiple species of *Aspergillus*. *Penicillium* metabolites were recovered from 82% of the samples, and *Alternaria* metabolites from 50%, reinforcing the hypothesis that the raisins are heavily contaminated with fungi. Ten samples were contaminated with metabolites associated with *Fusarium* spp. with the metabolites identified commonly associated with soilborne species of the genus. The fungal metabolite contamination suggests that major efforts are needed to improve the cleanliness of the raisin production process. With 99% contamination with *A. niger*, it is not surprising that many raisin exports from Afghanistan have high levels of ochratoxin contamination. The primary fungus that produces ochratoxin is nearly ubiquitous in its presence on these agricultural products.

3.4 Almonds. Six of the 89 almond samples were contaminated with more than 4 µg/kg of aflatoxins, with one sample contaminated at nearly 4,000 µg/kg. Two additional samples were contaminated at levels less than the 4 µg/kg EU cutoff, and two more contained precursors in the aflatoxin biosynthesis pathway, but no aflatoxin. None of the almond samples were contaminated with ochratoxin A, but three were contaminated with zearalenone at relatively low levels (< 100 µg/kg). *Alternaria* metabolites were identified in 65% (58/89) of the almond samples, while *Aspergillus* (30/89), *Penicillium* (22/89) and *Fusarium* (22/89) metabolites were all present in 25-30% of the samples. *Aspergillus niger* metabolites were present in only 10% of the samples, a result consistent with the lack of detectable ochratoxin A contamination.

3.5 Pistachios. Aflatoxin contamination was detected in 26/46 pistachio samples with 12/46 samples containing > 4 µg/kg of the toxin. In several cases contamination exceeded 1000 µg/kg with the highest level of contamination detected at 2,942 µg/kg of aflatoxin. Three of the samples that lacked aflatoxins contained aflatoxin precursors. More than 90% of the samples (43/46) contained *Aspergillus* metabolites and 40% contained *A. niger* metabolites. Thus most samples had been colonized by fungi that could produce regulated toxins under the appropriate storage conditions. *Penicillium* metabolites were found in slightly more than 50% of the samples (25/46) and *Alternaria* metabolites were found in 13 % of the samples.

3.6 Walnuts. The walnuts evaluated were very clean, with only 2/28 samples contaminated with low (< 0.8 µg/kg) of ochratoxin A and no aflatoxin contamination. All samples carried metabolites typical of infection with species of *Aspergillus*, but only four had *A. niger* metabolites, suggesting that the potential for contamination with ochratoxin A is relatively low as well. Around 2/3 samples were contaminated with *Penicillium* (19/28) or *Alternaria* (18/28) metabolites, and 40%

(11/28) carried evidence of *Fusarium* colonization. These levels suggest that hygiene in processing walnuts could be improved, and that a HACCP analysis of the chain could yield important insights into the best way to manage these nuts.

3.7 Wheat. Wheat is the major staple cereal in the Afghan diet, and studies of mycotoxins occurring on wheat in this part of the world are at best rare. The wheat cropping system is similar to that for other small grains such as rye, barley and oats, and differs significantly from that of maize where much more is known about the toxins, the spectrum of fungi that produce them, and the host plant's response to the producing fungus and to the contaminating toxin.

Much of the wheat grain in Afghanistan is generally dirty as indicated by the high percentage of samples contaminated with *Alternaria* (85%) and *Aspergillus* (66%). The presence of detectable levels of aflatoxin six samples is alarming, as aflatoxin contamination of wheat is not known to occur in the field and likely is the result of poor post-harvest storage procedures. *Aspergillus* species are usually soilborne and their presence indicates the grain probably has been in contact with the soil. *Alternaria* spp. usually are external colonizers that are removed when grain is processed.

Ochratoxins may be synthesized by species in the genera *Aspergillus* and *Penicillium*. Usually the *Penicillium* species are more commonly associated with ochratoxin production in small grains, and *Aspergillus* spp. are more commonly associated with ochratoxin production in products such as coffee, grapes, and cacao. *Penicillium* metabolites, including ochratoxins, were detected from 22% of the samples, with 12% containing detectable ochratoxins and 4% exceeding the EU guideline of 3 µg/kg. These numbers underestimate the increased exposure faced by many Afghans who consume relatively large amounts of wheat on a daily basis. Health problems in Afghanistan associated with ochratoxin contamination could include the relatively high levels of kidney failure, which is associated with consuming high levels of ochratoxin.

Fusarium metabolites were identified in 39% of the samples tested at BOKU, although only five contained detectable zearalenone, and only four contained detectable T-2 or HT-2. These levels suggest that *Fusarium* toxins were not a major problem in this year's crop, and that there are sufficient levels of inoculum available to potentially be problematic in at least some future years.

Contamination of wheat with ergot alkaloids was an unexpected problem. The relatively high percentage of samples containing ergot alkaloids (33%), including 6.5% with ergot alkaloids levels in excess of 100 µg/kg, suggests that an ergot epidemic occurred this past year in Afghanistan. The frequency of such epidemics is not well known. Ergot alkaloids are vasosuppressors and can restrict blood flow to the brain, causing hallucinations, and to body extremities – toes, fingers, feet and hands – resulting in tingling/fire as nerves are impacted, and then gangrene if blood flow is restricted for an extended period of time. Ergot also can infect numerous grasses and animals consuming pasture grass may become symptomatic as well as those consuming cultivated grains. Both the total amount of ergot alkaloids present and the relative frequency of individual ergot compounds can play a role in the severity of the effects associated with the consumption of the contaminated grain.

3.8 Non-regulated compounds. Many fungi produce secondary metabolites other than regulated toxins. These metabolites in some cases are known to alter (increase or decrease) mycotoxin production or the health impact of a toxin. Some detected secondary metabolites with known effects are listed in Table 7. Impacts of these compounds include immune system suppression (mycophenolic acid) and synergistic increases in kidney failure and kidney cancer when present with ochratoxin (citrinin).

Table 7. Additional secondary metabolites identified in samples associated with common producing genera.

Commodity	<i>Fusarium</i>	<i>Alternaria</i>	<i>Aspergillus</i>	<i>Penicillium</i>	Ergot
Wheat	Beauvericin	Tenuazonic acid	3-Nitropropionic acid	Mycophenolic acid	Ergocristine
	Enniatin A	Alternariol	Kojic acid	Agroclavine	Ergocristinine
	Enniatin A ₁	Alternariol methyl ether	Sterigmatocystin	Chanoclavin	Ergometrine
	Enniatin B	Tentoxin	Methoxy-sterigmatocystin	Elymoclavine	Ergometrine
	Enniatin B ₁	Altersetin	Averantin	Citrinin	Ergosin
	Epiequisetin	Altersolanol	Averufin	Secalonic acid D	Ergosinin
	Equisetin	Alttoxoin I	Norsolorinic acid	Questiomycin A	Ergotamine
	Chrysogin	Macrosporin		Quinolactacin A	Ergotamine
Nuts	Fusaric acid	Tenuazonic acid	Cyclopiazonic acid	Mycophenolic acid	
	α -Zearalenol	Alternariol	Kojic acid	Mycophenolic acid IV	
	β -Zearalenol	Alternario methyl ether	3-Nitropropionic acid	Penitrem A	
	HT-2 toxin	Altersetin	Asperfuran	Agroclavine	
	T-2 toxin	Tentoxin	Paspalin	Chanoclavin	
	Butenolid	Macrosporin	Nigragillin	Festuclavine	
	Epiequisetin	Infectopyron	Malformin A	Epoxyagroclavin	
	Equisetin		Malformin A ₂	Andrastin A	
Raisins		Tenuazonic acid	Malformin A	Mycophenolic acid	
		Alternariol	Malformin A ₂	Mycophenolic acid IV	
		Alternariol-methylether	Malformin C	Quinolactacin A	
		Altersetin	Pyranonigrin	Andrastin A	
		Alttoxoin-I	Nigragillin	Andrastin B	
		Tentoxin	Aurasperon B	Andrastin C	
		Macrosporin	Aurasperon C	Chanoclavin	
			Aurasperon G	Festuclavine	

3.9 Methodology evaluation.

3.9.1 ELISA test kits. The simplest technology available for testing for mycotoxins uses an ELISA (enzyme linked immunosorbent assay) test. These tests rely on an antibody binding to an antigen (the mycotoxin molecule) and the ability to discriminate an antibody bound to the antigen from one that is not bound to the antigen. These antibodies are the most labile part of a kit, and improper storage of the antibody can lead to kit failure and give either false positive or false negative results. ELISA assays are used in many research and testing arenas, but are not usually used for regulatory purposes. Instead, ELISA tests often are an initial screen that is used to identify potential problem samples that are then re-evaluated with more stringent chemical methods. ELISA methods often are specific for a single molecule and may or may not bind at the same or similar level to other related molecules that may be interconvertible with the molecule of interest. Chromatography and mass spectrometry assays usually do identify these related molecules. Developing, validating and calibrating commercial ELISA test kits is an expensive process, so before developing a kit, companies want to ensure that there is a sufficient market to warrant their up-front investment. Consequently, ELISA kits usually are available only for widely occurring mycotoxins on major crops. The assays are validated on different substrates from which the toxins may be recovered, and a test that works well with maize, for example, might or might not work well with another cereal such as wheat, rice or sorghum. Before being used on an alternate source, the manufacturer would need to validate it for use with that substrate. Each ELISA kit manufacturer must develop their own antibodies for use in their kit. The efficacy and properties of the antibody play an important role in the accuracy and reliability of the kit, and kits from different manufacturers may behave quite differently. The company with the best kit for one toxin may not necessarily produce the best kit for detecting another toxin. For logistic simplicity we used ELISA test kits from a single manufacturer, Romer Labs, for this study. Romer is a PHL partner and has a very good global reputation for leadership and reliability in diagnostic tests of mycotoxins.

Comparisons between tests and testing locations can be easily seen in Table 6 and Appendix 14. The shipping conditions to get materials to Afghanistan, the condition of the samples, and the operating conditions in the labs all could affect the efficacy of the ELISA tests. Shipment of test kits from the US to Afghanistan before the project started was via Dubai, and the shipment could have been exposed to excessive heat that affect antibody performance. There may be differences between treatment of grain, nuts and raisins in Afghanistan that could interfere with the efficacy of the antibody. Romer indicated that all of the tests were validated for the substrates we tested, but differences in processing, *e.g.*, use of sulfur in the drying process for turning grapes into raisins, could alter antibody sensitivity and explain some of the differences observed. No program should rely solely on ELISA tests for results. A back-up, such as the multi-mycotoxin screening done in Austria for this project, should always be included as part of a project's design and composition.

Something is different about wheat from Afghanistan. The Romer kit used in both the US and Afghanistan detected T-2 frequently and at relatively high levels, and the Neogen kit for T-2 tested in Nebraska with Afghan wheat was positive for every sample tested, even though chemically no T-2 was present in the samples. There also is variability in the ELISA tests for aflatoxin in wheat. The Neogen kit used in Nebraska matched the results obtained with the LC MS/MS assay. Romer

kits gave variable results, and the Vicam kit tested in Kansas gave a result that was completely atypical and resulted in the manufacturer temporarily suspending its validation of the kit for use on wheat until changes could be made.

The main point is that testing Afghanistan materials takes kits manufactured for first world use to their limit and exposes them to conditions under which they have not been rigorously tested. Ensuring that a kit provides robust results for Afghan materials is needed for large scale screens that go beyond research purposes. Including more extensive controls, including spiked samples and other known positive and negative controls, also would increase the reliability of the results obtained. Kit manufacturers are interested in these conditions as they want to increase the robustness and reliability of their kits and are willing to work with us to remedy these problems.

3.9.2 Sample collection and subdivision, and data management. Collecting and subdividing samples are the largest single source of variation in mycotoxin assays. Toxins are irregularly distributed within samples and this erratic distribution guarantees variation even in the most careful of surveys. Note that samples for regulatory purposes are much larger than those worked with in this study. Sample subdivision can be problematic if the subdivision is of large particles, e.g. individual grain kernels, nuts or fruits instead of a ground/homogenized sample. There were significant differences between results in Afghanistan with those obtained elsewhere. The groups in Nebraska, Kansas and Austria were all working with ground nut or wheat samples that were subdivided amongst the groups. The sample in Afghanistan was from the same original sample as the one worked with outside the country, but for nuts and raisins the subdivision occurred prior to grinding or homogenization. One explanation for differences would be if samples were “cherry picked” and those that were particularly good (or bad) looking were selected for analysis rather than a random selection. In this study the discrepancies observed in aflatoxins from raisins are perhaps the most noticeable and the most amenable to this explanation.

3.9.3 Data management. Data management can be challenging in studies such as this one as there are usually a large number of samples in different stages of processing, subdivision and analysis at the same time. This problem is noticeable in the data in Table 6, where different groups analyzed different numbers of samples. In addition, the common core of samples analyzed was not large (see Appendix XIV), and the set of samples analyzed at one location was not always the same as those analyzed at a second location. Results for the same samples analyzed at multiple locations can be seen in Appendix XIV.

4. Nominal Group Discussions

USAID together with MAIL and Kansas State University sponsored a meeting in New Delhi to discuss the results of the project and to begin the discussion with stakeholders in the public and private sectors, donors and international agencies in March 2016. Presentations were made on the results from the project and their interpretation, and on potential remediations and next steps follow-ups based on the data gathered thus far. Participants were divided into discussion groups and asked to consider a series of questions regarding the current status of and next steps for mycotoxin and food safety research in Afghanistan.

The Nominal Group technique was used to facilitate these discussions. This discussion process results in both a large number of ideas and a ranking of the most important of the ideas generated. Questions for consideration, instructions for discussion group functioning, and summarized raw responses by question may be found in Appendix I. The discussion below is taken from the New Delhi meeting report.

Discussions held by the meeting attendees were important for the diversity of the participants and the variation in points of view that were represented. Results are summarized by question and discussion section, and a complete set of responses and the guidelines given for the discussion process are attached. A more encompassing discussion of the results follows and contains some suggestions that could further food safety, especially with respect to mycotoxin exposure/contamination.

Technical Session Nominal Group Discussion

Question T-1 – Identify capacity building required for a sustainable mycotoxin surveillance program in Afghanistan.

The top two responses focus on communications and fundamental data acquisition and management. Public awareness is needed to aid data collection and a data repository is needed to discern patterns that may repeat over time and location.

The next set of responses are focused on having sufficient trained people to do the work and to be able to interpret the results obtained. Training was reflected in many responses further down the list as well, with various groups targeted for training and for particular topics that laboratory staff should be proficient to work with. “Appropriate” physical laboratories also are in this group. Identifying what an appropriate lab is varies as seen by responses further down the list, with descriptors such as accredited, quarantine, fee-based, multiple detection methods, outside Kabul, and in Kabul all included in responses.

Amongst the remaining responses that seem most significant were a need for appropriate governmental structure to deal with the issue(s), government funding for and recognition of the importance of the work, developing standard protocols to be followed, and efforts to help ensure people along the value chain from farmers to consumers were aware of issues and appropriate responses to problems that might occur.

Question T-2 – Identify data that should be collected to enable decisions regarding mycotoxin contamination to be made in Afghanistan.

Responses to this question often are not direct responses to the question asked, but instead are standards, protocols and processes for collecting the necessary data. Note that one response is for the use of an invalid technology for detecting toxin contamination.

More prominently mentioned data needed include GIS location, soil type, weather, variety grown, moisture content, *etc.* associated with a mycotoxin evaluation of a particular sample. Samples of data from along the value chain might help determine where mycotoxins are most likely to be increasing and to identify locations or conditions that are particularly problematic. Information on pre- and post-harvest conditions could be important, as could a more thorough evaluation of imports of potentially problematic foods.

Question T-3 – Identify ways to increase the credibility of the results obtained from mycotoxin surveillance surveys in Afghanistan.

Increased credibility was thought most likely to result if staff were better trained and training was an ongoing effort, and if the methodology being followed was both standardized and of international standard. There was a mixing of thoughts of whether labs had a research or a regulatory function, with public announcement of violations, enforcement of established standards, and laboratory accreditation of more importance if regulation is the lab's function. A visible commitment from the government to the effort and the availability of data to the public were also thought to be important incentives to increase the credibility of the work conducted.

Session 6 – Nominal Group Health and Trade Issues

Question 6.1 – Identify methods and goals for inter-ministry collaboration on problems associated with mycotoxins in Afghanistan.

At the top of this list is to define the roles and responsibilities for each ministry. Following a close second is to have regular meetings and to involve the private sector in those meetings. Clearly someone needs to own this issue and be in charge, however, and there cannot be a three-headed entity running the show. Thus, part of the definition of roles and responsibilities needs to include how the leadership issue will be managed.

Underlying the need for defined roles and responsibilities and effective communication is the development of aligned practices and guidelines. There are some specific suggestions for which ministry should be responsible for different tasks. All should have resources committed to the effort and all should have some role in establishing guidelines, regulations, monitoring systems, mitigation practices, and outreach to those outside the government. The relationship and the activities are likely to evolve with time, so building the system with enough flexibility to allow the evolution to occur is quite important as well.

Question 6.2 – Identify regulations needed to limit mycotoxin exposure in Afghanistan.

The most heavily weighted outcome was to establish maximum residual levels allowed in food and/or animal feed. Establishing regulations is best done by some sort of Food Safety Authority. This agency may need to be independent of the three ministries but have reporting responsibilities to all of them. Certainly coordination amongst the ministries and the Food Safety Authority will be essential. This agency could then be authorized to establish guidelines within various parameters, and could adapt guidelines and regulations as new information became available rather than waiting for legislative decisions on technical matters. Delegating responsibility for Food Safety issues that extend beyond mycotoxins should be a relatively straightforward process.

Responses past these initial high-ranked responses scattered in many directions, including particular places and situations where regulations should be enforced, how domestic and imported items should be treated, inspection processes for public and private labs, development of SOPs that go from farmers through to consumers, working conditions (especially security) for inspectors and other potentially targeted individuals in the regulatory process, and where the funding for the work to be conducted will come from.

Question 6.3 – Identify cultural barriers to be overcome to reduce mycotoxin exposure to mycotoxins in Afghanistan.

Responses to this question indicate that a significant study of how foodstuffs are managed by various groups in the country is going to be needed to help any proposed interventions succeed. Changes to traditional agricultural processes, food processing and food storage practices will be

especially important to implement in a careful and thoughtful manner. Dietary changes that reduce dependence on wheat as a staple food also may be difficult.

Beyond these major points many of the issues encountered may result from limited education of farmers and rural women who are responsible for much of the crop cultivation, food storage and food processing. Ensuring that communications come to them from a trusted authority is important and may be difficult to achieve.

Question 6.4 – Identify benefits resulting from lesser exposure to mycotoxins in Afghanistan.

The two top benefits identified were improved health and improved economic growth with more jobs. Perceived health benefits were reduced morbidity and mortality, less childhood stunting, improved productivity (as workers would not be out sick as often), and reduced costs from sending people outside the country for medical treatment. Increased health of domesticated animals could increase the availability of meat and other animal products as foods in the domestic markets.

Perceived economic benefits were quite numerous and most were not widely supported. They ranged from more food of better quality available in local markets to higher incomes for everyone along the value chain, and a better reputation (and price) for exports from Afghanistan with fewer rejections of exports as substandard. The ability for government ministries to work with each other and with private sector to reduce the problem would provide evidence that the government was doing something positive for the people and could open the doors to additional joint activities. A success of this sort would lift morale of many of those working in the food production business.

Session 8 – Nominal Group Food Safety and Security

Question 8.1A – Who needs information on mycotoxins in Afghanistan?

The basic answer to this question was everyone. At the top of the list were farmers, consumers, traders regulatory officials and extension workers. Some less obvious choices included on the list were health care providers, veterinary clinic staff and religious leaders. This question and question 8.2B are the only ones where every response was on at least one individual's "Top Five" list.

Question 8.1B – How should information on mycotoxins in Afghanistan be delivered?

There are many ways that information on mycotoxins could be delivered. The top three were public media (radio, TV, print, *etc.*), official government publications, and social media. These methods seem targeted at the broad consuming population as a whole. The remaining suggestions begin to fragment the population, with workshops and extension personnel ranking next. MAIL was the only ministry identified as needing to provide information, and that responsibility probably should be spread over all three ministries, but with differing target audiences.

Question 8.2A – When should screening for mycotoxins occur in Afghanistan?

Screening was envisioned as a routine thing for all commodities, with only one response suggesting that screening should be determined on the basis of environmental conditions. All but two responses suggested that screening should occur at harvest time or later, with processing, storage, market place and prior to export all receiving relatively strong support. Screening of materials to be imported was not ranked particularly highly.

Question 8.2B – Where should screening for mycotoxins occur in Afghanistan?

Some responses to this question are quite distinct from those to the “when” question (8.2A). The two most prominent locations were in the field prior to harvest and for imports at the border, followed by the marketplace and at the borders. Again there were a couple of responses suggesting that testing was needed at some times and not others, *e.g.*, “suspected locations” and “for crops at highest risk”.

Session 11 – The Future

For questions 11.1A and 11.1B, participants were asked to mark their top seven choices, instead of the top five, as was done with the other questions.

Question 11.1A – Identify priorities for the next year for research on mycotoxins and potential applications of solutions in Afghanistan.

Three of the top four priorities focus on government actions that can be started without significant scientific efforts. In particular, to establish an inter-ministerial/private sector task force (with a defined agenda and distributed responsibilities), to begin work to disseminate information to the general population, and to identify budget funds and show a commitment to work on mycotoxin reduction. Continuing the mycotoxin survey begun by this project was the fourth of the top priorities.

Education for MAIL staff and for exporters were the next most strongly supported activities. As with the first four activities, these activities could be seen as preparing groundwork for larger efforts in the future.

The remaining responses were quite scattered, and probably indicate the number of different directions that the work could take. I list below some of the ideas that seemed potentially the easiest to implement and where impact might easily be seen relatively quickly:

- Identify donors and other stakeholders and begin conversations with government ministries and private sector.
- Develop Good Agricultural Practices for Pre- and Post-Harvest management of crops.
- Begin analysis of value chains so that Critical Control Points in the HACCP process can be identified.
- Finalize food safety law and develop a series of SOPs for its implementation, including adopting limits on the most important mycotoxins.
- Adapt manuals (http://www.calpistachioresearch.org/GAP_Manual_2009.pdf) from the California Pistachio Research Board for local use. The main focus is on preventing fungal infections and subsequent mycotoxin contamination. There are numerous additional potentially useful links from the CPRB’s Home page that could be modified for use in Afghanistan. Some information already available at: http://afghanag.ucdavis.edu/a_horticulture
- Adapt GAP guidelines from the California Almond Board (<http://www.almonds.com/growers/growing-safe-product/gaps#harvest-delivery-sanitation>) for local use. These guidelines suggest food safety practices that extend far beyond concerns regarding mycotoxins. Some information already available at: http://afghanag.ucdavis.edu/a_horticulture. Similar information can be found for walnuts at: <http://www.walnuts.org/>. Information of this sort for raisin production in Afghanistan is already available on line (http://afghanag.ucdavis.edu/a_horticulture/fruits-trees/grapes).

Question 11.1B – Identify priorities for the next 5-10 years for research on mycotoxins and potential applications of solutions in Afghanistan.

Responses in this section are a continuation of those from the previous question. Many of the responses implicitly assume that many responses to 11.1A have been accomplished. Some responses are for continuation of these efforts, for instance Human and Institutional Capacity Development, the number 2 response, is going to be an ongoing process as will work on GAP and HAACP processes and protocols. By this time government funding should be firmly committed to the work, regulatory standards should be established, a functional Food Safety Authority should be in place, and the inter-ministry/private sector working group should be a routine activity.

Challenges awaiting this time frame are the accreditation process for public and private laboratories, a decision on whether the government should be involved in any way in “certifying” exports, enforcement processes for border inspections should be established, and at least some training of personnel to work in the area will be conducted by local experts. SOPs should be in place all along the value chains for the toxins relevant to those value chains, and a series of regional labs to provide quick tests should be in place around the country. If surveys have been conducted on an annual basis, then there should now be enough data to determine if there are any crop/geographic/weather hotspots for toxin occurrence patterns to be discernable.

Research questions will focus on agronomic and storage practices to reduce contamination, methods of mitigating contamination once it has occurred, and uses other than human food for materials contaminated with high levels of mycotoxins.

5. Recommendations for further work

5.1 Who should be involved

Mycotoxins are an interdisciplinary problem with questions and answers that span ministries. Thus, a collaborative approach to the problem is essential for long term success, and MAIL, MoCI and MoH all must be intimately involved in the work. As mycotoxins originate on agricultural products and are a problem in food and feed chains, MAIL is best positioned to lead future efforts in the area of mycotoxins and should be specifically charged with doing so. MAIL should organize and chair an inter-ministerial working group in this area as soon as possible

Training efforts are needed at multiple levels within Afghanistan. Current USAID projects provide contacts and venues for training Extension Agents and farmers. Materials to be used for training on mycotoxins and food safety/security should be developed in collaboration with these existing programs and should include topics such as Good Agricultural Practices, Good Storage Practices and Good Manufacturing Practices. These efforts should mesh with the proposed risk communications training to develop outreach materials and to identify core messages. The Afghanistan tertiary education sector also should be engaged in these efforts through external training of staff at BS, MS and PhD levels, and the development of laboratories where research in these areas can be conducted.

USAID should foster the following interactions: Development of a center of excellence for mycotoxins in the region. Tajikistan currently is a Feed the Future partner country and might be the best target for such activity. US and European academic and/or governmental labs should provide support. The Center of Excellence would provide training needed for staff at all of the regional labs and would be charged with analyzing data on a regional basis to identify places with (or at high risk) of contamination in the crops. A good model for this Center of Excellence lab would

be the mycotoxin-focused programs of BecA, located on the ILRI campus. The center of excellence proposed here would have a focus on small grains and problems in central, southern and southwestern Asia, which are not a part of the mandate for the BecA effort. Such a center's long-term viability requires visibility, a reputation for excellence and relevance, and support from multiple donors.

5.2 Awareness and Risk Communications

Public awareness of the impact of mycotoxins problems in trade, agriculture and health was a major theme of the Delhi meeting, as reflected in the nominal group discussions. Risk communications and strategies to raise public awareness with raising public alarm were the subject of a subsequent meeting organized by the USAID mission in Kabul on 16 July 2016 (Appendix XV). Awareness comes in various forms and formats and must be distributed along the value chain from farmer to consumer. The needs of ministry employees who help manage the problem are different from those of traders and private sector actors who buy/sell and import/export agricultural goods which are different again from university staff/students conducting research in this area and differ even further from those of the farmers or the general population in the city and in rural areas. Raising awareness is critical and must be done in a manner such that those who hear the message are energized to address the problem in a positive manner and not are so frightened that they freeze up and nothing happens. A project to address this topic will be complex due to the different target audiences, the development of specific materials and messages for each group, and the diverse platforms through which information can be delivered (Table 8). A proposal for a project to begin developing risk communication skills within the three ministries is included as Appendix XVI.

Afghanistan does not have a history of deaths or other severe debilitations tied explicitly to a mycotoxin, *e.g.*, aflatoxins in Kenya or fumonisins in South Africa. The approach at this time should be towards better post-harvest storage practices, increased food quality, and care and cleanliness in food preparation, and to avoid raising "boogeyman" type issues that could discredit the government, lead to panic/fear in one or more of the target groups, or distort the marketplace as one or more foods is avoided for potential food safety reasons.

MAIL, MoPH and MITC must collaborate to establish common themes and priorities. MAIL should communicate with farmers, MAIL and MITC should jointly communicate with traders, importers and exporters, and MoPH and MAIL should jointly communicate with consumers and the general public. Such efforts require commitment from the highest levels in the ministries to the tasks and buy-in from those working in the middle levels of the ministries for the desired outcome(s) to occur. An important first effort could be training sessions on the inter-relatedness of the issues for ministry staff. The training could take many different forms, but there must be enough team-building for staff from all three ministries to be working on collaborative, rather than competitive, approaches, solutions and endpoints. USAID and other external parties may need to assist with this training as the number and depth of trained personnel available within the Government of Afghanistan is very limited.

Farmers need to understand their role as conditions before and during harvest can have a major impact on the amount of mycotoxin contamination in items entering the food system. Training in Good Agricultural Practices is the single most important thing that could be done to reduce mycotoxin contamination in Afghanistan. Incorporating background information on the detrimental

effect of these compounds into the GAP training is probably the easiest way to get this information to farmers. GAP training can occur in many different ways. SWABO (Scientific Animations Without Borders), through the Post Harvest Loss Innovation Lab, has developed numerous cell-phone based training modules and games that have been well received in other developing countries and have been used successfully in conjunction with more traditional outreach programs. Traders and importers/exporters need to know that mycotoxins can reduce the value of the items they are buying and selling. In the case of exports, mycotoxin contamination can not only affect the price, but also may affect whether a product can be sold at all, or must be destroyed at the exporter's expense. That different export markets have different sensitivities to mycotoxin contamination needs to be more generally known and could open up new markets that could be more easily penetrated than those of the European Union, whose regulations are the strictest in the world.

5.3 Capacity

Afghanistan needs to develop the capacity to manage mycotoxin contamination locally. Physical and human capacity both are currently limiting. Physical capacity includes appropriately equipped laboratories with 24-hour electricity and secure storage for reagents and samples, as well as appropriate means for disposing of contaminated samples and hazardous materials generated during the analytical process. Human capacity requires staff with both specific training in particular activities and general training in mycotoxins and associated activities. Assessments of both physical and human capacity for doing the work need to be assessed by a combination of internal and external personnel. The capacity assessment should include government ministries, universities and the private sector. External partners should be identified to assist in training and to provide critical technical backstopping.

A plan for work to be conducted can be developed once the human and physical capacities have been assessed and should complement the increasing human and physical capacities. Appropriate SOPs for the analysis(es) being conducted must be developed and implemented, and a process to validate results and estimate errors established. Continued external assessment of the the government, academic and private sector capacity should continue and be conducted in a manner that honestly evaluates the credibility of the results reported. Developing credible laboratory capacity for research and information purposes should be possible in governmental, university and private settings.

Developing credible laboratory capacity that could be used for regulatory purposes might be possible for a private laboratory, but the culture of power and corruption associated with government agencies will make developing credible regulatory capacity much more difficult in a government setting. For regulatory purposes, a better approach would be to establish regulations and develop the capacity to accredit laboratories, rather than to simply have laboratories in which work is conducted be accepted simply because these labs are government run. The capacity to accredit laboratories for their ability to assess food safety could be extended far beyond mycotoxin analyses and would be a significant government service for the country as a whole.

5.4 Medical assessments

Public health measures *per se* were not a major focus of this project, but are an important component of addressing mycotoxin contamination problems. The extent to which individuals have been exposed to various toxins is important to understanding the mitigation steps that should be taken.

Biomarker assays using both blood and urine are becoming available for many toxins. These protocols require medically trained personnel to conduct studies as part of an interdisciplinary team looking at the overall food availability and food security problems in the country. Additionally, the Afghan diet needs to be rigorously documented in a series of “food basket analyses. Based on the foods consumed, exposure levels to toxins can be determined and guidelines for safe consumption of foods established. This process is particularly important since the Afghan diet is disproportionately high in wheat. Contamination levels that are acceptable internationally may not be acceptable in Afghanistan due to differences in diet.

5.5 Beyond Mycotoxins

Much can be done in terms of food safety that goes beyond mycotoxins. Both chemical and biological, primarily microbiological, hazards exist. Including mycotoxin work within this broader food safety context probably is essential for sustainable research and regulation of mycotoxins.

A second area worthy of further research is the effect of fungal secondary metabolites beyond mycotoxins on human and animal health. There are numerous secondary metabolites that are not toxic in and of themselves, but certainly can impact human health. In this survey citrinin and mycophenolic acid were detected and these compounds can alter immune system activity and kidney function, respectively. Some of the unknown causes of these problems may be related to synergistic interactions with mycotoxins or other secondary metabolites. Little work is done in this area, and could be very important as the emphasis of research shifts from acute mycotoxicoses to assessing the results of chronic exposure to contaminated foodstuffs.

5.6 Government of Afghanistan ministries interests in further work

Appendix XVII contains summaries of discussions held as part of the 16 July 2016 meeting held at the USAID mission in Kabul on Risk Communications. These interests largely parallel those outlined in this report. MAIL has the broadest interest and is viewed as the agency to be the primary coordinator for future efforts. MoPH indicated low priority for work with mycotoxins due to numerous other competing issues viewed as having higher priority. MoCI views mycotoxins as having high priority for imports and moderate priority for exports. All indicate that education and training are needed for individuals within the ministries and for the general Afghan public that they serve.

A few specific comments on these plans are warranted.

MAIL – GAP should be the initial focus, as many problems could be reduced with better and cleaner handling of various agricultural products both in the field and postharvest. Implementing GAP is probably the single most cost-effective action that could be taken. Developing an AflaSafe/AflaGuard product for use in Afghanistan will probably take longer than the 6-12 months envisioned here. Such a product could be very useful for nuts. Work in this area should be coordinated with MoCI to ensure that nuts produced in orchards using the biocontrol would be acceptable for export. Additionally, the biocontrol approach may be useful in preventing toxin levels from increasing in storage of already harvested nuts. For grapes/raisins/sultanas the main focus should be on ochratoxin A. Systems that could be used for biocontrol of this toxin are poorly developed and not yet proven in commercial settings. Collaborative research with groups outside the country would be essential to develop a product that could be used for these products.

For MoCI, a decision is needed on the role of the ministry in exports of food products. Providing information and guidance to producers as they choose export destinations is quite different from regulating where goods can be shipped. For imports, guidelines and enforcement policies

need to be developed that enable rapid, transparent tests of target foods and food products. There is a note that MoCI has laboratory facilities that could be used for testing purposes. An assessment of the capacity of this lab is included as Appendix VI.1. Revisiting this assessment and enabling collaboration between this lab and the MAIL lab is important.

For MoPH, the primary issue is to build education and communication capacity on this topic and to ensure that information on mycotoxins is incorporated into appropriate staff training and public information materials. Should human health problems that are directly attributable to mycotoxin contamination occur in the country, MoPH will certainly be one of the ministries that becomes quickly involved in the situation. Thus, they need an established response plan that involves both communication with the general public as well as a process to pass primary responsibility for managing a crisis to appropriate staff within MAIL.

5.7 Specific Recommendations for further activity

1. MAIL, MoCI and MoH – Implement Risk Communications training program for ministerial staff. (short-medium term).
2. MAIL – Implement grain sorting and cleaning program with GAP, GSP and GMP for wheat and wheat products. Include training across entire value chain. Develop sorting/cleaning equipment by Asiatic mills and subsistence farmers. Develop non-food uses, e.g., as a fuel source, for materials not qualifying as food grade. (short-medium term)
3. MAIL – Multiple year sampling and mycotoxin surveys of wheat and other foods at research level, not regulatory. (short to long term)
4. MoCI and MAIL – Develop regulations and diagnostic capabilities to enable rejection of low quality imports, particularly wheat. (short term)
5. MoCI and MAIL – Develop resources to enable Afghan exporters of nuts and dried fruit to target export countries on the basis of mycotoxin contamination levels. Training of private sector staff to conduct mycotoxin assessments for internal use. (short-medium term)
6. MoH and MAIL – Evaluate local Afghan “food baskets” and measure biomarkers for mycotoxin exposure in urban and rural Afghan populations. (short to medium term)
7. Ministry of Education and MAIL – Use internal and external evaluation teams to assess capacity of Afghan universities for instruction in food quality and safety. Develop target curriculum for one or perhaps two universities. Enable external degree training (BS, MS & PhD) to staff selected universities for teaching, research and outreach in these areas. (short to long term)
8. USAID – Spearhead efforts to develop a regional center for excellence for mycotoxins in collaboration with other donors and US and European research entities. (short to long term)

Table 7. Strategic design of a project to increase Afghan government capacity to communicate risks associated with mycotoxins and other food safety issues.

	Producer	Trader	Processor	Distributor	Exporter	Importer	Government	Health/Vet	Consumer
Technical Experts							✓	✓	
Highly Literate	✓	✓	✓	✓	✓	✓	✓	✓	✓
Average Literate	✓	✓	✓	✓	✓	✓	✓	✓	✓
Nominally Literate	✓	✓	✓						✓
Illiterate	✓								✓
Training							x	x	
Technical Info	x	x	x	x	x	x	x	x	x
Public Education	x								x
Advocacy		x	x	x	x	x	x	x	
Crisis Commun	x	x	x	x	x	x	x	x	x
Health Risk	High	Moderate	Moderate	Low	Moderate	Low	Low	Low	High
Financial Risk	High	Moderate	Moderate	Low	High	High	High	Low	Low
Relevant Deliverables	3,4,6,7	3,4	3,4	3,4	2,3,4	2,3,4,	1,4,5,8,9,10	2,3,4,5,6,7	3,4,6,7

Risk – High, Moderate, Low

Potential deliverables from a project to increase awareness of mycotoxins. Kansas State University, working in conjunction with the USAID Office of Agriculture (OAG) and the Development Outreach and Communications Office (DOC), will arrange for a strategic communications advisory team to provide communications guidance and capacity building support for MAIL based upon strategies developed by MAIL to increase the safety of Afghan agricultural products for export and consumption. All of the information provided will be available in English. Portions of the information provided should be available in Dari, and Pashto, languages in which K-State lacks the expertise necessary to provide qualified translations both literally and in terms of the cultural context within the country. Areas for guidance and capacity building support may include but are not limited to:

1. Guidance on the development of a strategic communications plan based upon strategy developed by MAIL to increase the safety of Afghan agricultural products for export and consumption. Plan to include guidance on methodology and timelines for initially informing relevant audiences and for keeping these audiences informed as the implementation of MAIL's strategy progresses, and benchmarks that can be monitored and evaluated;
2. Guidance on monitoring domestic and international traditional media and social media concerning mycotoxins and MAIL's ability to address the problem(s);
3. Polling of target audiences over the course of the implementation of the project to track changes in opinion and understanding of relevant issues;
4. Guidance on the organization of press conferences, media roundtables, meetings and town halls at the national, provincial, and community level to inform and educate the general public, farmers, medical and veterinary professionals, and agribusinesses on how to increase the safety of Afghan agricultural products. To be delivered and/or simultaneously translated into English, Dari, and Pashto;
5. Guidance on the advance drafting and translating of talking points, press releases, updates, and social media toolkits to be shared by MAIL and other Afghan government stakeholders;
6. Guidance on the production of radio and television Public Service Announcements to be broadcast across the country;
7. Guidance on the oversight of social media communications related to these issues on MAIL and other Afghan government social media platforms;
8. Guidance on the identification of relevant stakeholders in the government and private sector in export markets for Afghan products to include Afghan embassy officials, foreign government representatives, and foreign private sector representatives and the organization of meetings with these stakeholders;
9. Training for MAIL communications staff on best practices for government health- and safety-specific emergency communications;
10. Guidance on the development of a crisis communications plan for future events that can be utilized by MAIL.

All communications products and event organization will be developed in conjunction with MAIL to ensure that the Ministry communications team develops the capacity to implement a crisis communications plan in the future without outside support.

Appendix I – Scope of Work

Scope of Work
for the
Rapid assessment of Mycotoxins in Afghanistan's food value chains

I. PURPOSE

The purpose of this assignment is to assist USAID/Afghanistan and the Government of the Islamic Republic of Afghanistan's (GIROA) Ministry of Agriculture, Irrigation and Livestock (MAIL) undertake a rapid assessment of the prevalence of mycotoxins in the staple wheat food and high value horticulture value chains. The assignment will be implemented under the USAID Bureau for Food Security's Leader with Associate mechanism for the Innovation Lab for the Reduction of Post-Harvest Loss (PHL Innovation Lab, the Awardee).

The PHL Innovation Lab will provide the technical expertise to design and implement the assessment, but do so in a collaborative manner with MAIL. The PHL Innovation Lab will be responsible for preparing a final report that will summarize findings, and propose recommendations for follow-up actions which should include ways to strengthen the institutional capacity of Afghan government and private sector entities to address mycotoxin contamination if it is identified as a problem.

II. BACKGROUND

Mycotoxins are metabolites (by-products) of the growth of molds. They have toxic side effects to plants, animals, and humans. Mycotoxin contamination of crops has been a worldwide problem for thousands of years. Only in the last thirty or forty years has technology allowed researchers to isolate the fungal mycotoxins and study the effects on feed crops, livestock, and their effects on humans.

Aflatoxins are naturally occurring mycotoxins that are produced by several *Aspergillus* species of fungi, the major ones are *Aspergillus flavus* and *Aspergillus parasiticus*. The *Aspergillus* genus of fungi produces toxins that exhibit a wide range of toxicities, with the most significant effects being long term. Aflatoxin B₁ is a potent liver carcinogen. Ochratoxin A and citrinin both affect kidney function. Clycopiazonic acid has a wide range of effects and tremorgenic toxins affect the nervous system.

In August 2014 when the concept paper for this assessment was drafted by the USAID/Afghanistan Office of Agriculture (OAG), the office did not have definitive information regarding mycotoxins in Afghanistan's food chain; however, there is circumstantial evidence to raise concern. The OAG's research revealed the following:

Link between cancer and mycotoxins. According to a study conducted by researchers at the University of Pittsburgh (see annex), "*Hepatocellular carcinoma (HCC), or liver cancer, is the third leading cause of cancer deaths worldwide, with prevalence 16–32 times higher in developing countries than in developed countries. Aflatoxin, a contaminant produced by the fungi *Aspergillus flavus* and *Aspergillus parasiticus* in maize and nuts, is a known human liver carcinogen. Of the 550,000–600,000 new HCC cases worldwide each year, about 25,200–155,000 may be attributable to aflatoxin exposure. Most cases occur in sub-Saharan Africa, Southeast Asia, and China where populations suffer from both high HBV*

prevalence and largely uncontrolled aflatoxin exposure in food. Aflatoxin may play a causative role in 4.6–28.2% of all global HCC cases.”

Link between crops and mycotoxins. Correspondence with the Head of the Plant Pathology Department at Kansas State University, Dr. John Leslie, noted, “*First, I think that aflatoxins and ochratoxins are both potential problems on the tree nuts and dried fruits. Both are made by Aspergillus fungi. Aflatoxins would be associated with liver problems, while ochratoxins could be associated with the kidney problems, as they are best known as nephrotoxins. The wheat problems could be aflatoxins, but the storage has to be pretty awful for it to be an issue there. A more common problem on wheat would be toxins produced by Fusarium fungi. Which toxin and which fungus depends a bit on the climate where the wheat was originally grown. Aflatoxin and ochratoxin could be a problem in spices as well as the tree nuts and dried fruit. Checking them for quality could be important as well. If peanuts/groundnuts are grown in Afghanistan, then there could be contamination problems with them as well.*”

Incidence of liver and kidney cancer in Afghanistan. According to World Health ranking (<http://www.worldlifeexpectancy.com/cause-of-death/liver-disease/by-country/>), Afghanistan ranks 6th in the world for liver cancer and 3rd for kidney cancer.

The circumstantial evidence led the OAG to pose the problem statement: *Is there a prevalence of mycotoxins in Afghanistan’s food value chains that contributes to the high levels of liver and kidney cancer?*

In October 2014, during a meeting between OAG and the Deputy Minister for MAIL, the Deputy Minister revealed that aflatoxin is indeed a problem that had adversely affected Afghanistan’s horticultural exports to Europe. Rejection letters from European buyers provided clear evidence that mycotoxin contamination is a problem; however, the scope of the problem is not self-evident. The Deputy Minister expressed concern that neither MAIL staff nor donors were responsive to the problem.

III. SCOPE OF WORK

The PHL Innovation Lab will assist counterparts from the MAIL to undertake a rapid assessment exercise for identification of mycotoxins in the wheat and high value horticulture export value chains. Because mycotoxins have already been identified in some exportable commodities like grapes and pistachios, the PHL Innovation Lab and MAIL experts will work to ascertain the scope of the problem.

This assessment will generate primary data on the key indicators to be maintained and updated in a database populated jointly by USAID and MAIL. This data can be used to help in the design of follow-up activities supported by MAIL, private sector and donor community.

Another important outcome of the assessment will be to strengthen the capacity of MAIL’s technical staff to design and implement similar assessments after USAID’s assistance has ended. MAIL’s leadership views building the capacity of its staff and associated institutions of paramount importance, especially as the GIRoA strives to lessen its dependency on the international donor community.

The PHL Innovation Lab is expected to address and undertake the following key activities and tasks summarized below:

1. Design a research and sampling methodology

The PHL Innovation Lab, prior to inception of field activities, will develop the approach and methodology for conducting the assessment basing it on international best practices and standards.

2. Training of field staff in use of sampling technology

Central to this investigation will be the use of low-cost, rapid assaying kits that are used to detect mycotoxins. There are several commercial suppliers of these kits (e.g. <http://www.elisa-tek.com/diagnostic-testing-kits/mycotoxins/>) and the PHL Innovation Lab will select the most appropriate for the proposed task.

The assessment will be undertaken in key market and production nodes (e.g., wheat flour depots, packing houses) and should be coordinated in conjunction with MAIL. Depending upon the technology deployed, MAIL staff may have to be trained by the PHL Innovation Lab.

3. Implementation of data collection

The PHL Innovation Lab will work with MAIL staff to sample and assay commodities located in various parts of the country. MAIL staff time and facilities will serve as the GIRA's in-kind contribution to this assessment project.

4. Technical & material support to MAIL

To complement building technical capacity within MAIL, the activity will also provide support for equipment and supplies deemed essential by the PHL Innovation Lab in order to continue mycotoxin research and detection beyond the life of this project.

5. Draft report & presentation to MAIL, MoCI, MoPH, and WFP

The PHL Innovation Lab will draft progress reports and a final report to be shared with MAIL, MoCI, MoPH, and WFP.

6. International Workshop on pre and post-harvest loss reduction

The PHL Innovation Lab will provide administrative and technical support for organizing an international workshop in support of addressing pre- and post-harvest losses with a special emphasis on Afghanistan. This workshop will take place outside of Afghanistan near the end of this project.

IV. SUGGESTED METHODOLOGY

The PHL Innovation Lab will be expected to perform the tasks in a systematic manner and develop a detailed methodology at the outset of the assignment. The process applied to the assignment shall be

consultative and participatory. The findings should be validated at various stages of the project. The PHL Innovation Lab will work with MAIL staff throughout the project area to collect required information, analyze and compile data.

The suggested methodology which will be revised together with the PHL Innovation Lab is briefly described below:

Document Review: The PHL Innovation Lab will review all relevant available data/reports related to the tasks. In addition to reviewing documents, briefing materials will be provided to MAIL. MAIL will facilitate access to relevant data sources within Afghanistan and provide international data sources it has available to the PHL Innovation Lab.

Consultation with MAIL and other GIROA ministries: The PHL Innovation Lab together with project staff will develop a tentative list of stakeholders to be consulted with for each set of activities. Additional individuals may be identified by the PHL Innovation Lab at any point during the project.

Design of Research approach: The PHL Innovation Lab will design and produce a questionnaire in close consultation with the Project M&E staff and that will be given to the project field staff prior to the study.

Field sampling: The PHL Innovation Lab will conduct field visits to selected program areas in all regions where required. The PHL Innovation Lab will also conduct selected visits to representatives and key stakeholder in the value chains. During these visits representative samples of the crops of interest will be collected for mycotoxin analysis.

Information Collection and Analysis: The PHL Innovation Lab with the help of the project staff will collect in different regions of interest representative samples of wheat and other high value horticulture products for mycotoxin analysis. Samples will be evaluated using appropriate technology for mycotoxin quantification. . The PHL Innovation Lab will also review existing data collected by the project staff.

Reporting. The PHL Innovation Lab will provide a PowerPoint briefing of their major findings and/or important next steps with MAIL and USAID before their return to the USA. A final report will be sent to USAID no more than one month after the conclusion of field work.

V. DELIVERABLES

Detailed Methodology and Action Plan: The PHL Innovation Lab will develop a detailed work plan and a brief suggested methodology to be reviewed and approved by project management. This should be done no later than the end of first month of mobilization.

Progress Update: The PHL Innovation Lab will compile a brief progress update on a bi-weekly basis using a report template agreed to by sponsor and awardee prior to project start.

Final Report: The outline of the report will be developed in the inception phase. Both hard and soft copies of the reports prepared in MS-Word will be provided along with relevant literature reviewed.

Stakeholder Briefings: A final briefing will be held by the PHL Innovation Lab for MAIL. USAID representative/s and key stakeholders will be present to reflect on the major findings and recommendations.

MAIL Lab support. In addition to providing training to MAIL staff, this project provides support for the equipping of MAIL labs to help with the continuation of mycotoxin research and detection. The PHL Innovation Lab will identify, select and order the equipment to be purchased for conducting the mycotoxin analysis of the field samples collected. MAIL staff will be trained on the use of this equipment, and that equipment will remain in the MAIL labs after this project is concluded.

International Workshop in 2015. USAID will circulate the results of this investigation among development partners with the intent of prompting further research and appropriate measures to improve food quality and reduce post-harvest losses. The Awardee will organize an international workshop on post-harvest losses and food quality towards the end of this project where the results and mitigation recommendations will be presented. This workshop will take place outside of Afghanistan in the summer of 2015.

VI. ILLUSTRATIVE DURATION, TIMING AND SCHEDULE

Task	LOE	Estimated Schedule
Pre-project scope of work development and initial research methodology development	4 weeks	January 1-30, 2015
Desk study. Review available reports related to assignment	1 week	February 2-5, 2015
Consultation with MAIL, USAID other stakeholders via conference calls	1 week	February 9-13, 2015
Development of research methodology	3 weeks	February 16-March 6, 2015
Confirmation of approach and preparation to undertake assessment	8 weeks	March 9-May 1, 2015
Procurement of assay kits	2 weeks	May 4-13, 2015
Departure for Afghanistan	2 days	May 14-15, 2015
Training of assayists and sample collectors	1 week	May 16-21, 2015
Sample collection along supply chains	4 weeks	May 23-June 18, 2015
Lab Sample Analysis	4 weeks	June 20—July 16, 2015
Preliminary Analysis of data	2 weeks	July 18-30, 2015
Pre-departure briefing	1 day	July 29, 2015
Finalization and submission of report	2 weeks	August 3-14, 2015

Support for organizing international workshop	4 weeks	August 17- September 11, 2015
International Workshop	2 days	September 14-15, 2015

Appendix II – Modification #02 AID-OAA-L-14-00002

ASSISTANCENO.
AID-OAA-L-14-00002

MODIFICATION NO.
02

11. DESCRIPTION OF MODIFICATION (CONTINUED)

Islamic Republic of Afghanistan's (GIRoA) Ministry of Agriculture, Irrigation and Livestock (MAIL) undertake a rapid assessment of the prevalence of mycotoxins in the staple food (specifically, wheat) and high value horticulture value chains. The activity will be implemented under the Bureau for Food Security's Leader with Associate mechanism for Post-Harvest losses.

Mycotoxins are harmful metabolites (by-products) from the growth of molds. They have toxic side effects to animals and humans. Mycotoxin contamination of crops has been a worldwide problem for thousands of years. Only in the last thirty or forty years has technology allowed researchers to isolate the fungal mycotoxins and study the effects on feed crops, livestock, and their effects on humans.

In October 2012, during a meeting between OAG and the Deputy Minister for MAIL, the Deputy Minister revealed that aflatoxin (a class of mycotoxin) is indeed a problem that had adversely affected Afghanistan's horticultural exports to Europe. Rejection letters from European buyers provided clear evidence that aflatoxin contamination is a problem supports the proposed assessment.

Activity Purpose

The technical consultants will provide necessary expertise to design and implement the assessment, in a collaborative manner with MAIL, in order to identify mycotoxins in the wheat and high value horticulture export value chains. Data generated from the assessment can be used to help in the design of follow-up activities supported by MAIL, private sector and donor community. In addition, the assessment would include ways to strengthen the institutional capacity of Afghan government and private sector entities to address mycotoxin contamination.

The consultants are expected to address and undertake the following key activities and tasks summarized below:

1. Design a research and sampling methodology based on international best practices
2. Train MAIL field staff in usage of sampling technology
3. Implement data collection: The consultants will work with MAIL staff to sample and assay produce located in various parts of the country. MAIL staff time and facilities will serve as the GIRoA's in-kind contribution to this assessment.
4. Generate progress and final report which will summarize findings and propose recommendations for the follow-up actions
5. Support the organization of an international workshop on post-harvest losses.

3) Accounting Data:

Accounting Template: 306-KABUL-SOAG-FY2012
BBFY: 2011
EBFY: 2015
FY: 2012
Fund: ES
OP: AFGHANISTA
Prog Area: A26
Dist Code: 306-M
Prog Elem: A140
Team/Div: AFG/OAG
BGA: 997
SOC: 4100100
Obligation: \$1,220,535.00

Account Template: 306-KABUL-SOAG-FY 2010
BBFY: 2011
EBFY: 2012
Fund: ES
OP: AFGHANISTA
Prog Area: A26
Dist Code: 306-M
Prog Elem: A140
Team/Div: AFG/OAG
BGA: 306
SOC: 4100100
Obligation: \$927,480.00

ALL OTHER TERMS AND CONDITONS REMAIN THE SAME.

Appendix III – Initial project training materials

III.1 Training video list for MAIL staff

III.2 Mycotoxins Overview presentation

III.3 Mycotoxin Vale Chain Assessment Project presentation

III.4 Sampling Procedure Protocol presentation

IV.5 Sample code key

Accurate and Reliable Testing of Mycotoxins in Agricultural & Food Products <https://www.youtube.com/watch?v=J7kut5N3ubw>

Risk Assessment Related to Pathogenic Hazards in Food Processing <https://www.youtube.com/watch?v=2lTh2PAaIE>

PH Meter زاهج استخدام قيرط (You will be using this) <https://www.youtube.com/watch?v=BVbKcQTZIKs>

PH موهفم <https://www.youtube.com/watch?v=KCQuaua8hJQ>

Micropipetting (You will be using this) <https://www.youtube.com/watch?v=NgosWmRjjAo>

Multi-channel pipette: Important points when using (You will be using this) <https://www.youtube.com/watch?v=Irp80f9RVtQ>

Pipette Calibration and Cleaning (You will be using this) <https://www.youtube.com/watch?v=MBq55FtOzN4>

Measurement Uncertainty and Calibration Tolerances <https://www.youtube.com/watch?v=Zy1kt6EKOWI>

How to calculate a serial dilution (You will be using this) <https://www.youtube.com/watch?v=HZzpgjGosmg>

Concentration of Solutions: PPM and PPB Parts Per M/B https://www.youtube.com/watch?v=Wzj_TL95-Q

Concentrations Part 1 <https://www.youtube.com/watch?v=V11BtOOOrRY>

Concentrations Part 2 <https://www.youtube.com/watch?v=yeRzphpG1O4>

Concentrations Part 3 <https://www.youtube.com/watch?v=yFn59OMUqOU>

Concentrations Part 4 https://www.youtube.com/watch?v=a3_NmawmxKM

Concentrations Part 5 - serial dilution https://www.youtube.com/watch?v=ZqdU3VfQ_Tc

Concentrations Part 6 <https://www.youtube.com/watch?v=0kD68RCnypQ>

Filtration **(You will be using this)**

<https://www.youtube.com/watch?v=Q0s71cjCNWs>

Spectrophotometry - Finding the concentration of an unknown **(THIS IS VERY IMPORTANT SINCE THIS IS HOW THE EQUIPMENT WE WILL BE USING WORKS)**

<https://www.youtube.com/watch?v=NRGA8XMNR5I>

Determining the Concentration of an Unknown Sample Using the Standard Curve Excel 2010 **(also important)**

<https://www.youtube.com/watch?v=1BdVmIATl2w>

Beers Law **(also important)**

<https://www.youtube.com/watch?v=4GI-6uR8k4o>

Spectrophotometric Enzyme Assays

https://www.youtube.com/watch?v=egiBP_fPnBA

ELISA Tutorial 1: How a Direct, Indirect and Sandwich ELISA Works

<https://www.youtube.com/watch?v=nNjIBCnpGZ4>

ELISA Tutorial 2: Coating and Blocking the ELISA Plate

<https://www.youtube.com/watch?v=AmG7FBolfdc>

ELISA Tutorial 3: Preparing and Adding Samples to the ELISA Plate

<https://www.youtube.com/watch?v=darrx6F0wsg>

ELISA Tutorial 4: Finishing the Assay (Sandwich ELISA)

<https://www.youtube.com/watch?v=zI4khIjCd8>

ELISA Tutorial 5: Preparing ELISA Data in Excel for Analysis with GraphPad Prism

<https://www.youtube.com/watch?v=I9tO81ZCeRg>

ELISA Tutorial 6: How to Analyze ELISA Data with GraphPad Prism

<https://www.youtube.com/watch?v=5lqqpKSnXfl>

Mycotoxin MycoSep Columns **(You will be using this)**

<https://www.youtube.com/watch?v=3QBkCLZlvDU>

Bioser S.A. - Kit AgraQuant de Romer Labs **(You will be using this)**

<https://www.youtube.com/watch?v=g4LtXpgLtSY>

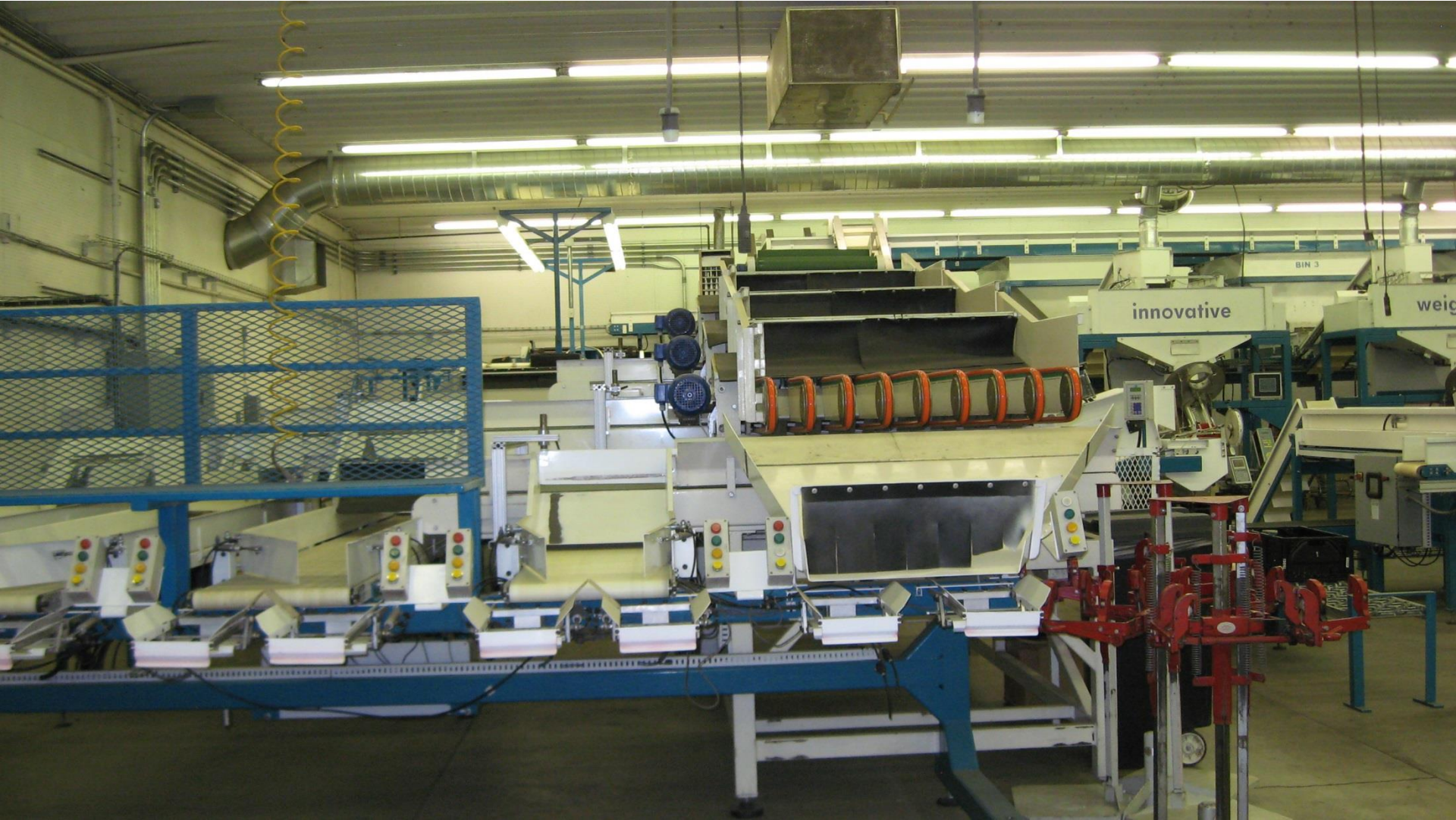
AgraQuant Mycotoxin ELISA **(You will be using this)**

<https://www.youtube.com/watch?v=pzUj6gxuy3g>

AgraStrip® WATEX Training Video **(You will be using this)**

<https://www.youtube.com/watch?v=54PmQqtNC4>







Mycotoxins: An Overview

Andreia Bianchini, PhD

University of Nebraska - Lincoln

and

Debra Frey, MSc

Kansas State University



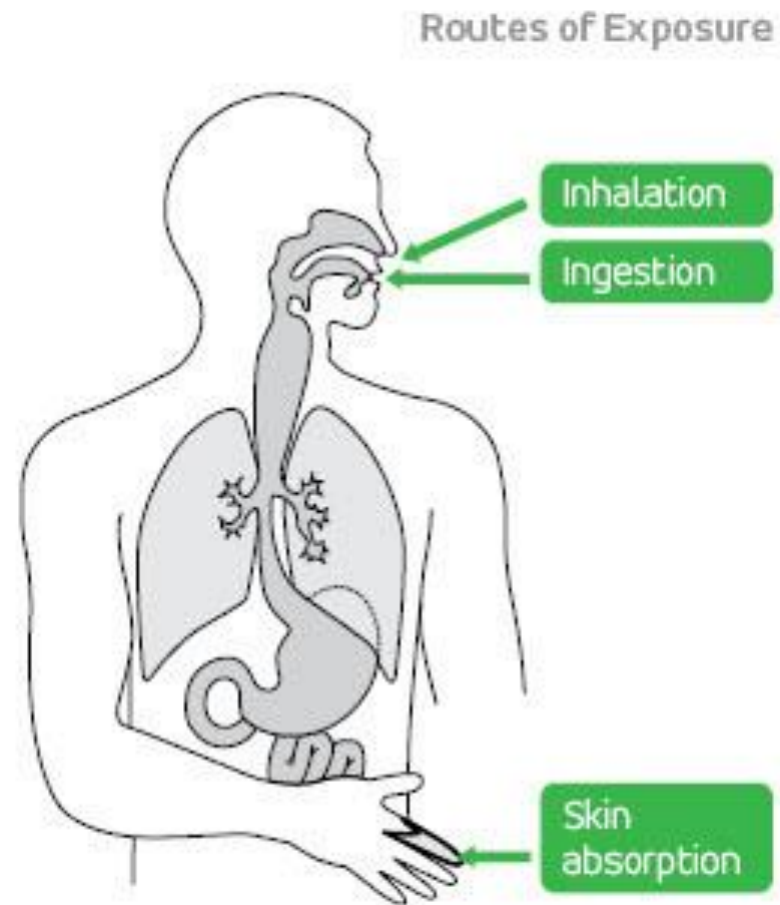
General Information

- Large, diverse group of fungal toxins
- Naturally occurring
- Toxic to plants, animals, humans, microorganisms and cell cultures
- May be thousands of unique mycotoxins in nature
- True number is unknown



Exposure

- Ingestion (Direct or Indirect)
- Inhalation
- Direct dermal contact



Effects of Mycotoxins

- Acute exposure
 - Vomiting/Gastrointestinal diseases
 - Death

- Chronic exposure to insidious low-levels
 - Growth retardation and lack of weight gain
 - Impaired immunity
 - Tumor formation



Concerns About Mycotoxins

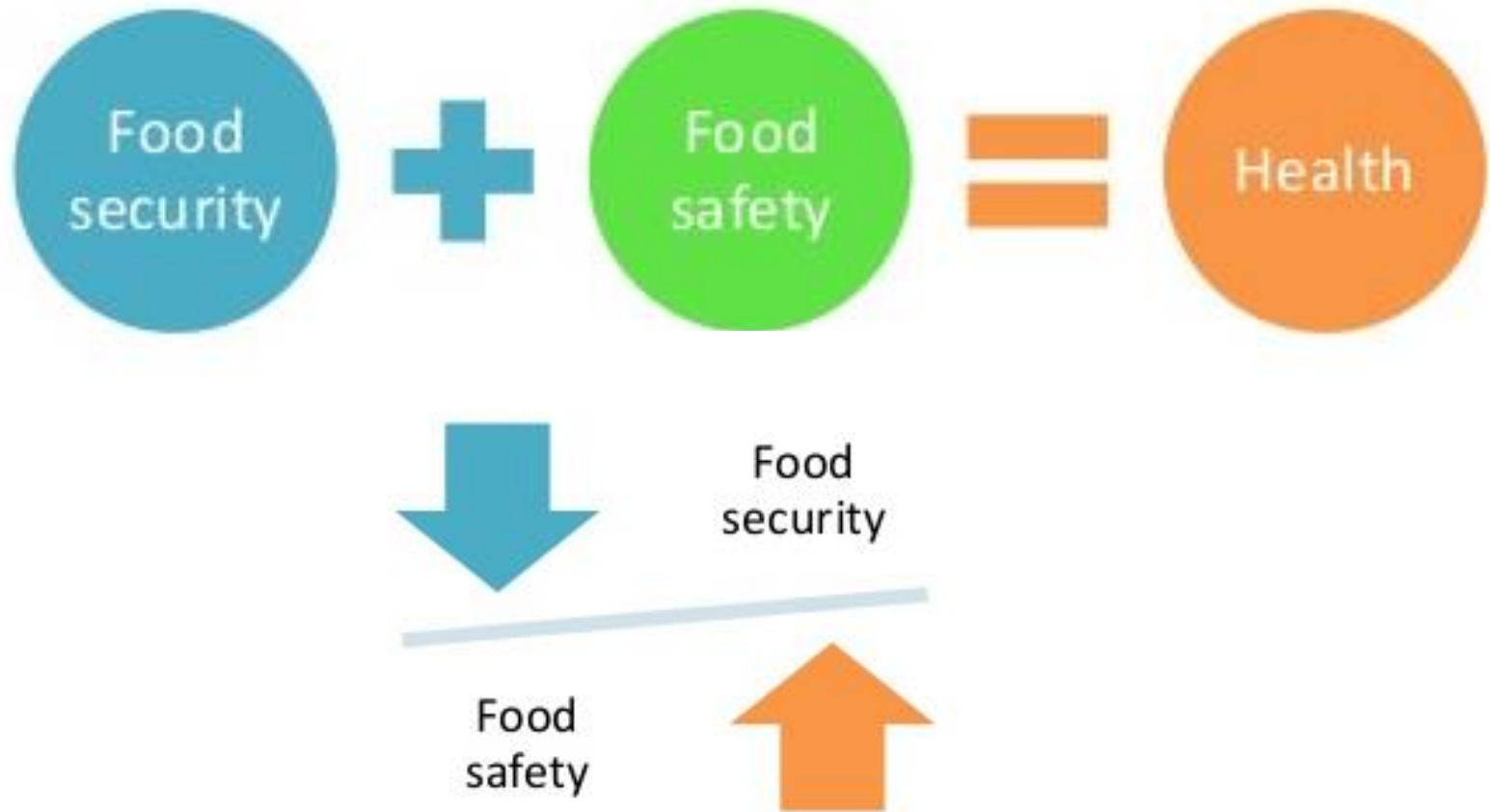
- Where populations have a single dietary staple
 - May be exposed to great amounts
 - Acute and chronic toxicity possible
 - Less developed countries – more direct exposure

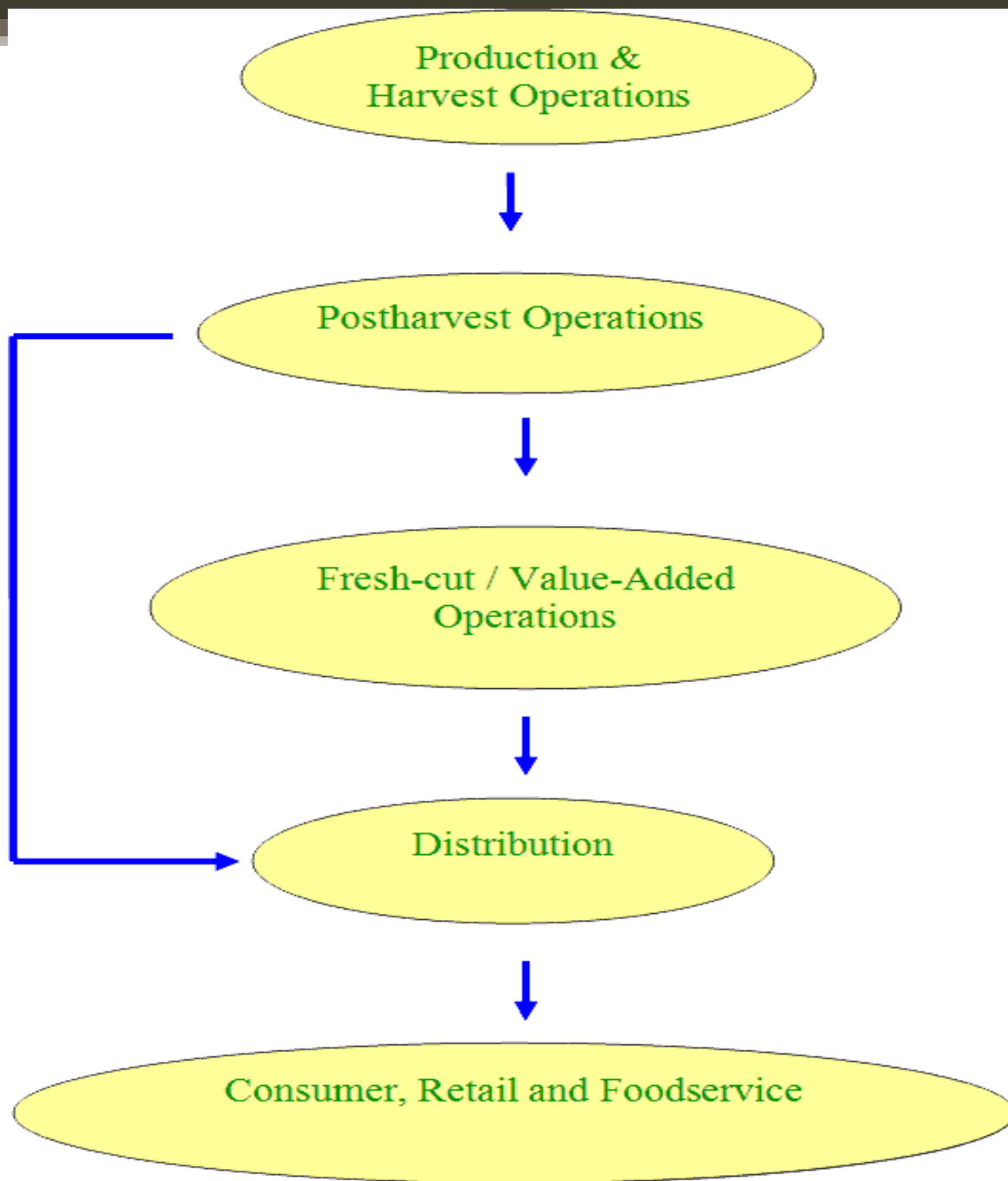


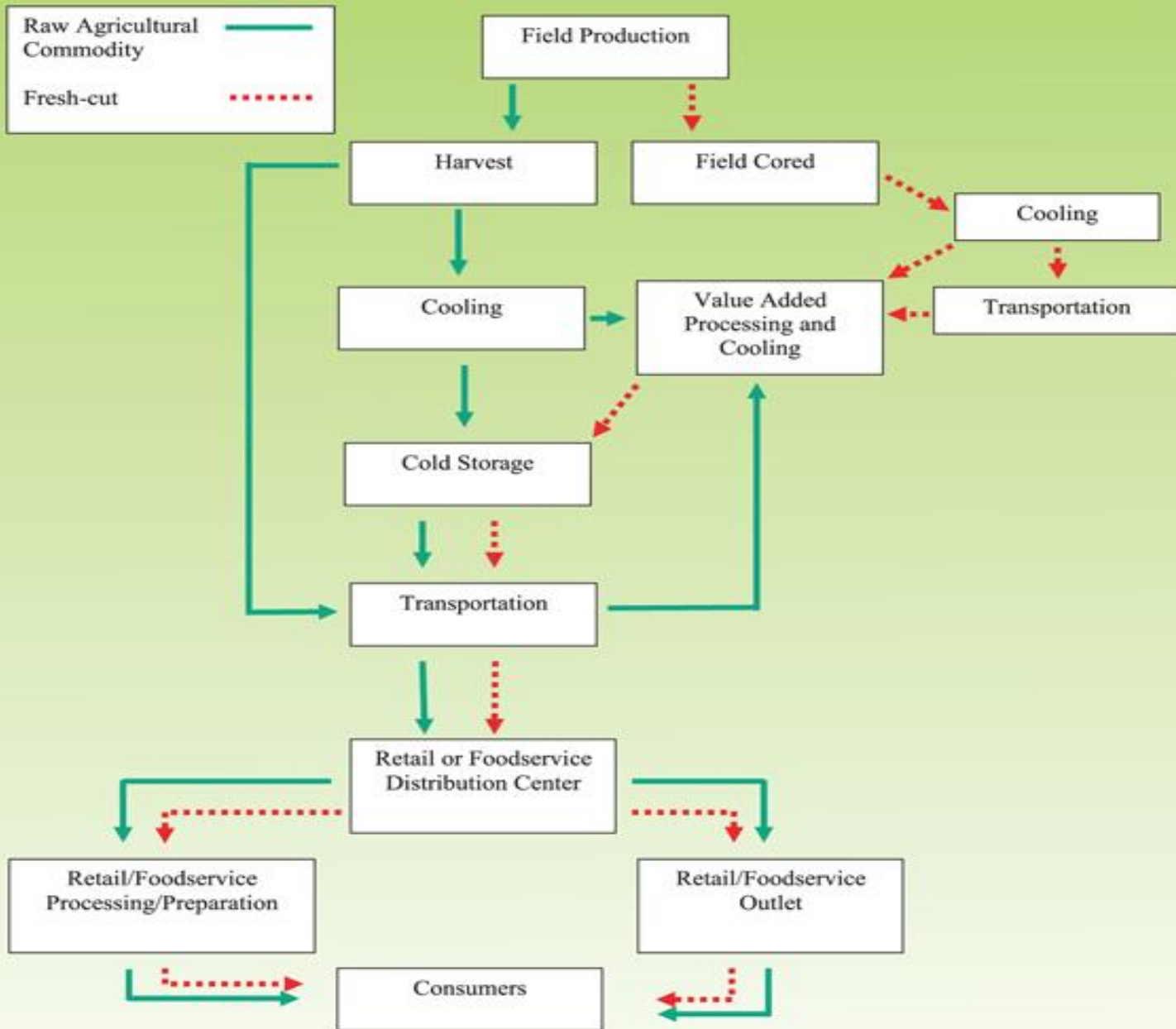
- Where diets are diverse
 - Low levels of exposure
 - Foods of better quality – lower amounts
 - More developed countries – direct and indirect exposure
 - ❖ Food Ingredients
 - ❖ Residues in animal products – milk, eggs, edible organ tissues



Mycotoxins: a multi-disciplinary issue







- **REGULATORY ACTION GUIDANCE:**
- The following represents the criteria for direct reference seizure to Division of Compliance Management and Operations (HFC-210) and for direct citation by the District Offices:
- **NOTE:** Examine a minimum of 10 subs from each code or from the lot if no codes are present.
- **MOLD:** Natural raisins average 5 percent or more by count moldy.
- **SAND:** The average is 40 milligrams or more of sand and grit per 100 grams of natural or Golden Bleached raisins.
- **INSECTS:** The following represents the criteria for recommending legal action to CFSAN/Office of *Compliance*/Division of Enforcement (HFS-605)

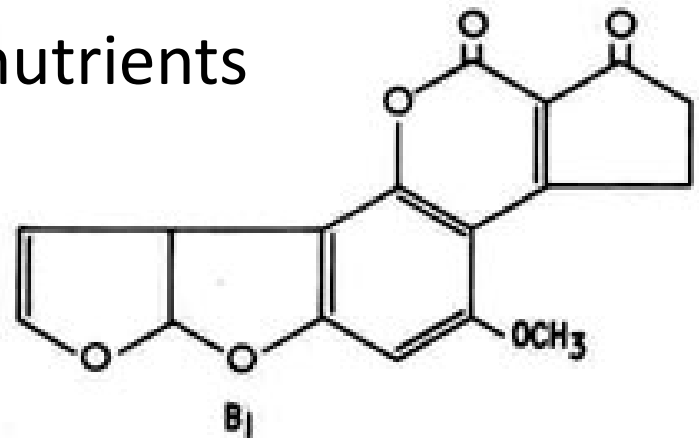




11.01.2015 08:48

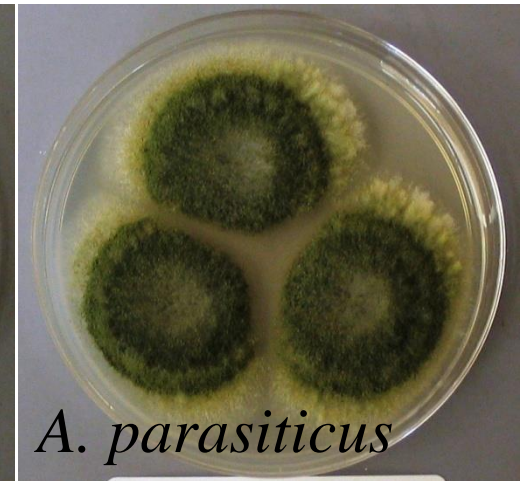
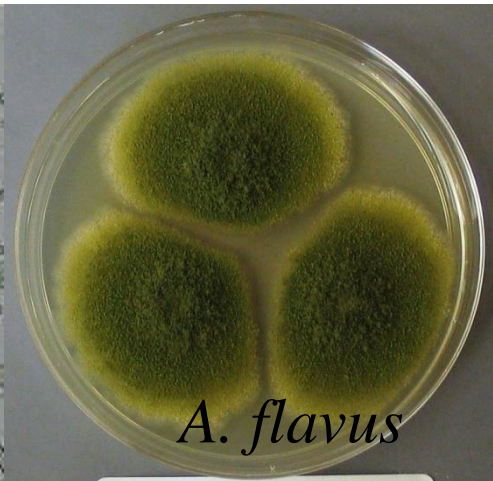
Aflatoxins

- Aflatoxins are heptocarcinogens
 - Involved in human liver cancer worldwide
 - Liver cancer is most prevalent in the tropical regions of the world (where toxin mostly occurs)
 - May cause cancer in other organs and tissues
 - Aflatoxin B₁ is most toxic and most carcinogenic
- Immunotoxic
- Interferes with absorption of nutrients
 - Stunting in Afghanistan



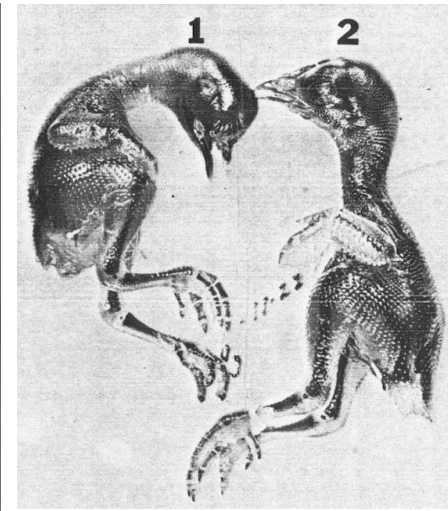
Aflatoxins

- Produced mainly by *Aspergillus flavus* and *A. parasiticus*
- May contaminate cereals and oil seeds
 - Wheat, raisins, dried fruit, corn, peanuts, tree nuts, and cottonseed



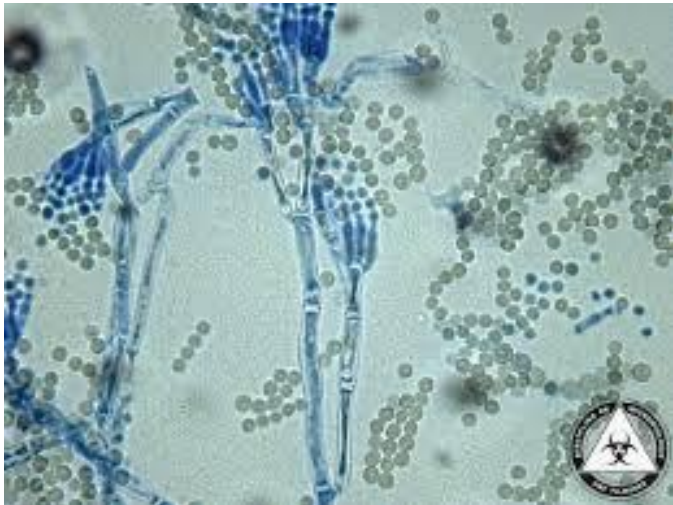
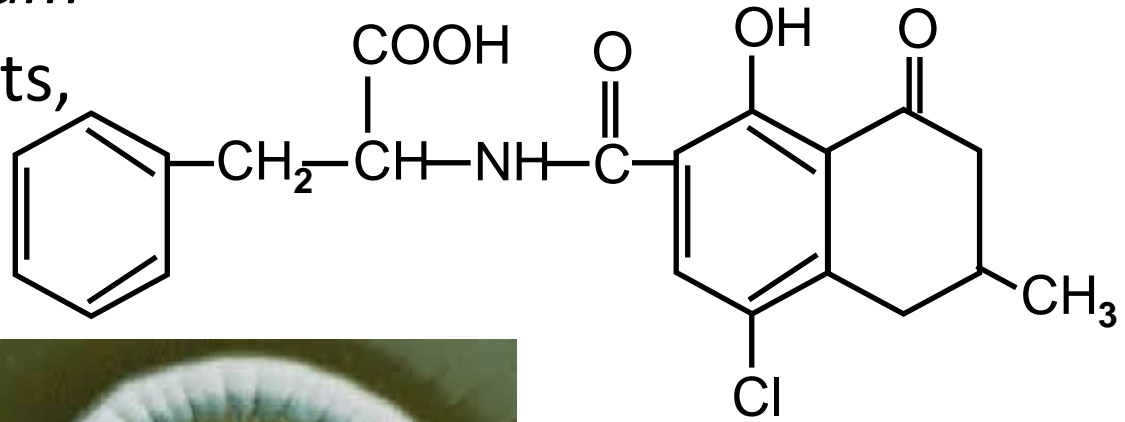
Ochratoxin A

- Nephrotoxic – kidney damage
- Carcinogenic to kidneys, embriotoxic, and teratogenic
- Diseases associated with ochratoxin A:
 - Porcine Nephropathy
 - Balkan Endemic Nephropathy



Ochratoxin A

- Produced by *Aspergillus ochraceus*, *A. carbonarius*, and *Penicillium verrucosum*
- May contaminate nuts, raisins and wheat



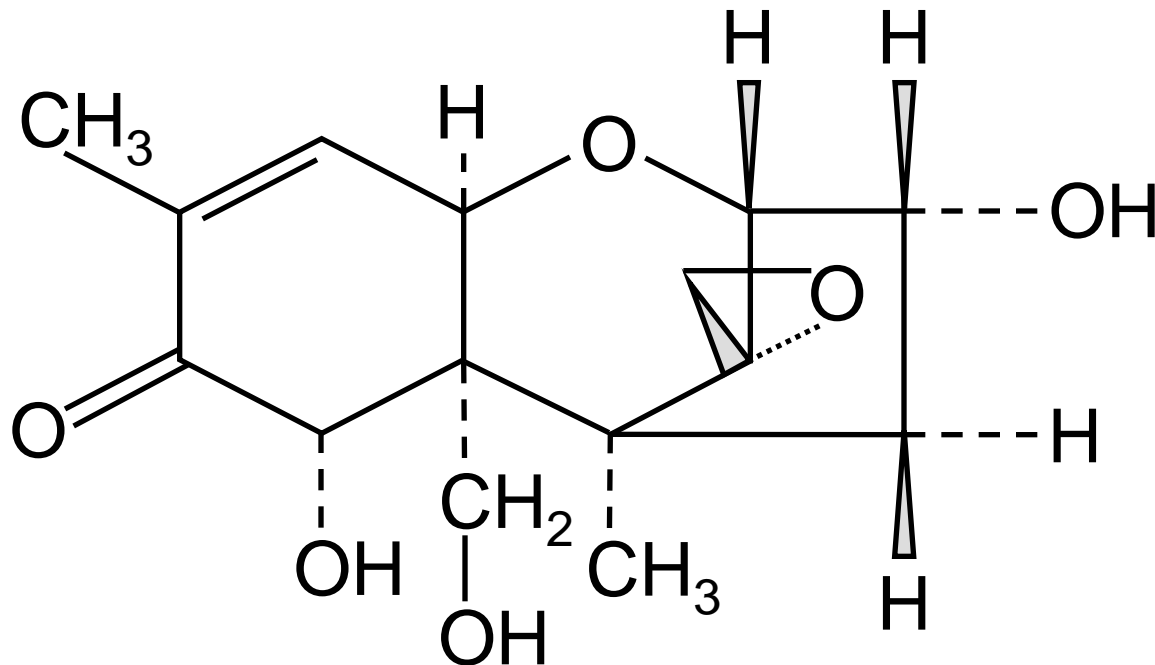
Deoxynivalenol/Vomitoxin

- Causes vomiting or emesis in cattle, dogs, cats, and humans
 - Vomitoxin
- Causes foodborne illness or gastroenteritis in humans
 - Nausea, facial rash, throat irritation, abdominal pain, diarrhea, headache, fever, chills
- Suppress immune system



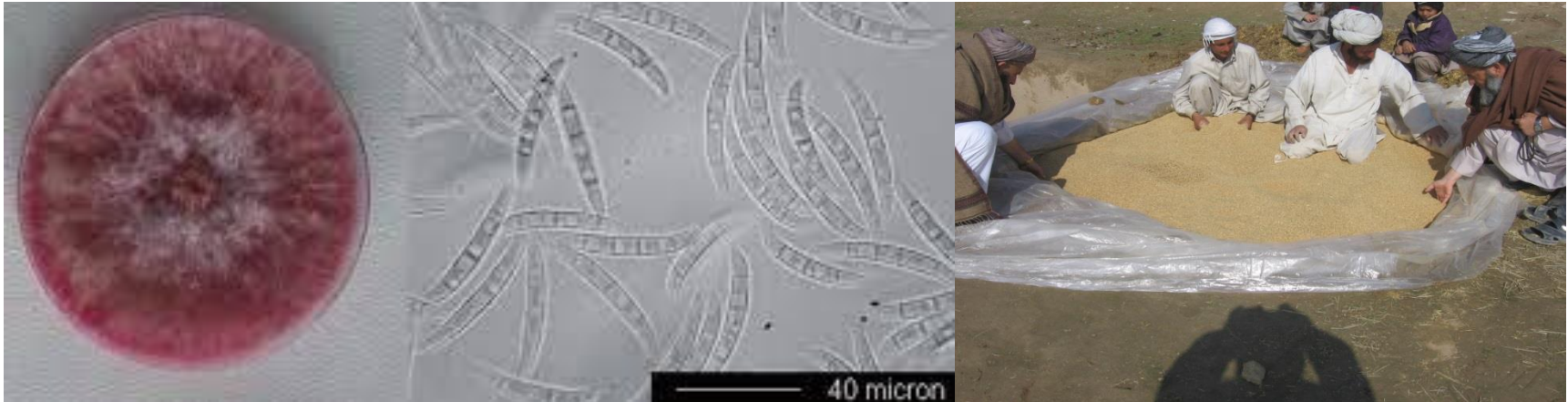
Deoxynivalenol/Vomitoxin

- Produced by *F. graminearum* and *F. culmorum*
- One of a group of mycotoxins known as trichothecenes
- Found in diseased grain (i.e. corn, wheat, barley)



Fumonisin

- Diseases associated with fumonisins:
 - Cause severe immunological or hematological problems, therefore representing contaminants of considerable concern to human and animal health.



Fusarium sp.

- Trichothecene (T2) Mycotoxin
- In humans, it has been linked to:
 - Esophageal cancer in South Africa, Northeast Italy and Northern China
 - Neural tube defects in developing human embryos
 - The most dangerous of the mycotoxins
- Mainly found in wheat



Mycotoxins of Greatest Concern in Grains and the Molds that Produce them

Mycotoxins

Aflatoxins

Ochratoxin

Fumonisin

Deoxynivalenol
(DON, Vomitoxin)

Molds

Aspergillus flavus, A. parasiticus, A. nomius

Aspergillus ochraceus, A. niger
Penicillium verrucosum

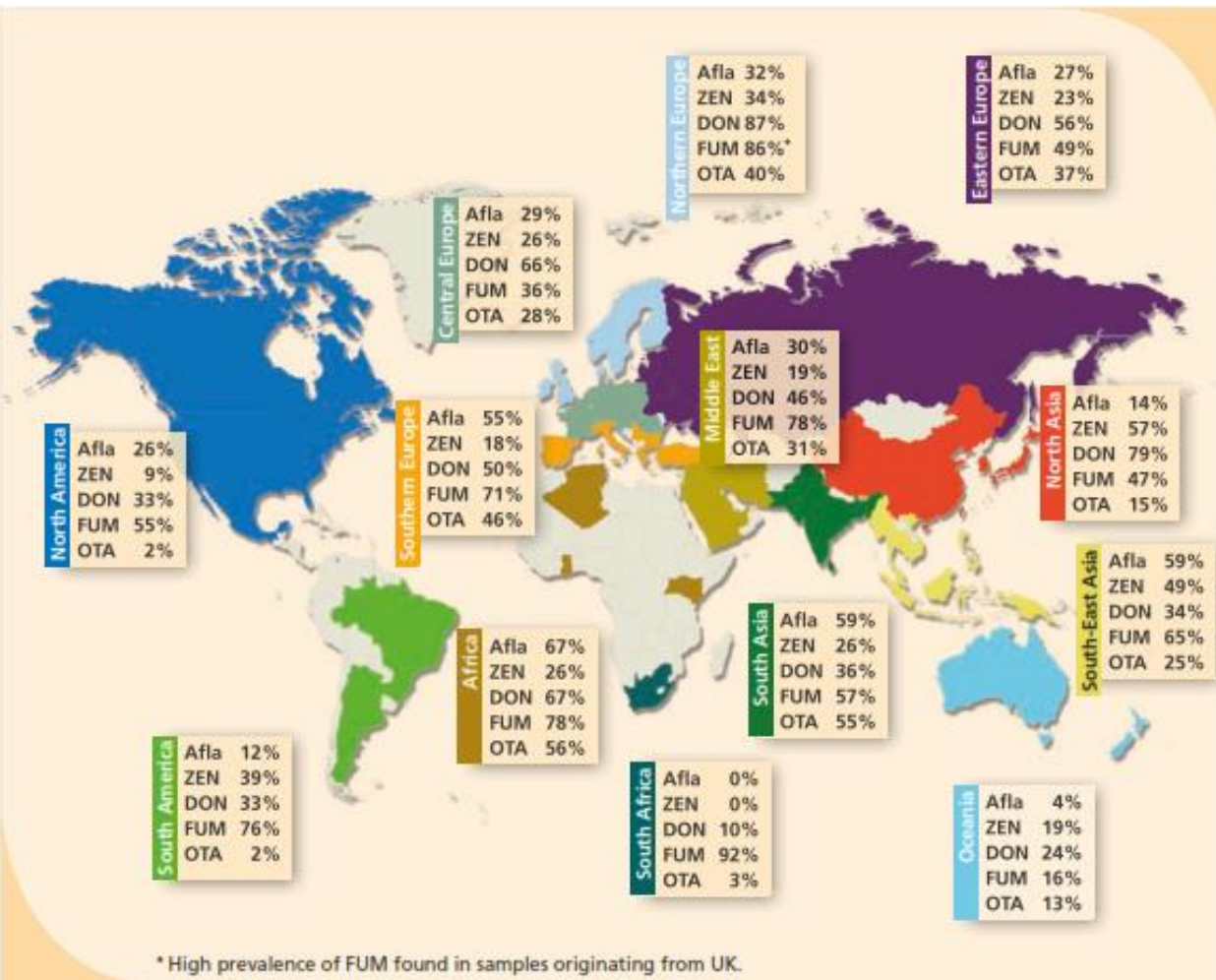
Fusarium verticillioides (moniliforme)
F. proliferatum, F. subglutinans, F. tricinctum

Fusarium graminearum, F. culmorum
F. pseudograminearum

Geographic Pattern of Mycotoxin Occurrence

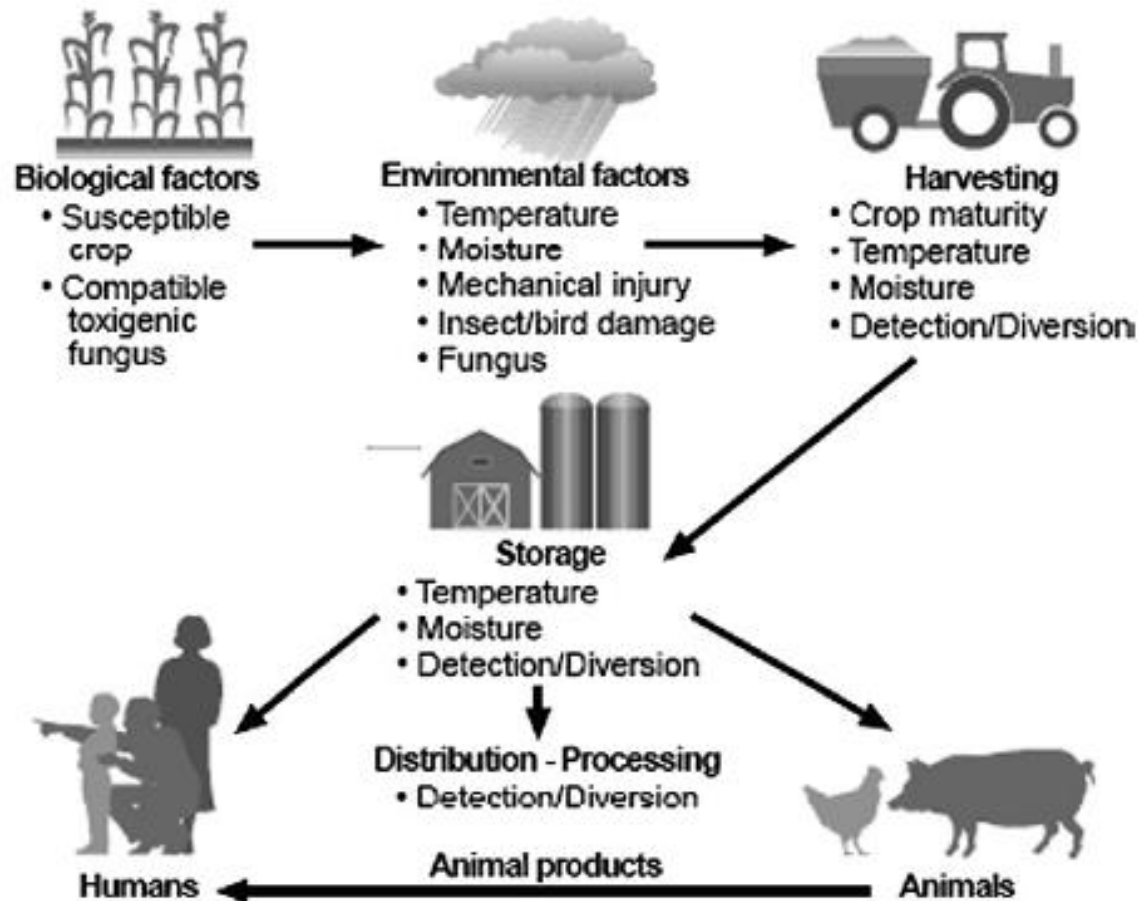


Mycotoxin Global Occurrence in 2013



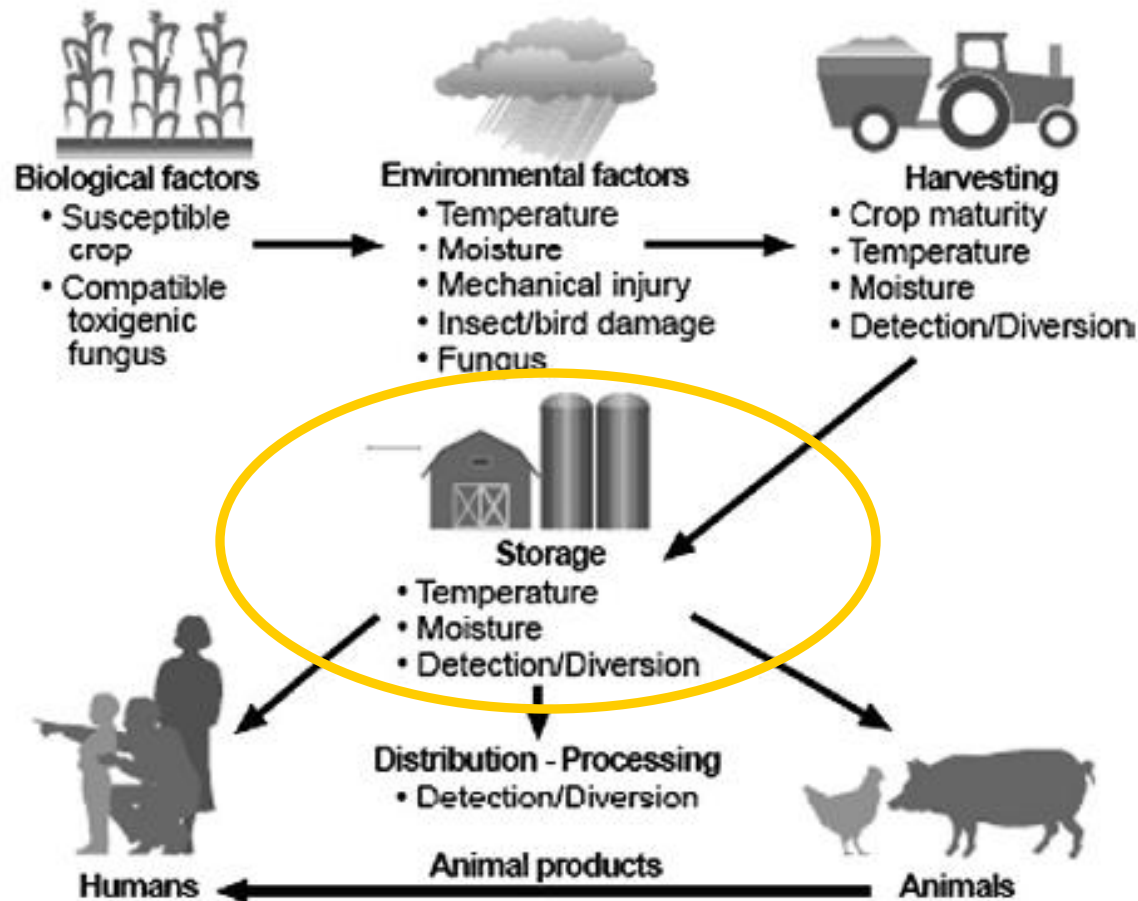
- On average, in the >4,200 samples:
 - AFLA: 30%
 - ZEA: 37%
 - DON: 59%
 - FUM: 55%
 - OTA: 23%

Mycotoxin Occurrence in the Food Chain



Factors affecting mycotoxin occurrence in the food chain (CAST, 2003).

Mycotoxin Occurrence in the Food Chain



Factors affecting mycotoxin occurrence in the food chain (CAST, 2003).

Mold in Grain - Storage Problems

- High humidity and moisture problems (14-30%)
- Warm temperatures (25-35°C)
- Fluctuating and low temperatures
- Extended storage time
- Insect and mite activity in the grain
- Main molds of concern:
 - *Aspergillus*
 - *Penicillium*
 - Fumonisin

INSPECTION AND ASSESSMENT OF GRAIN WAREHOUSE



Control Measures During Storage

- Dry grain properly before storage (below 12-14%)
- Provide good aeration of the grain
- Treat grain to kill insects
- Monitor insect activity
- Avoid extended storage time

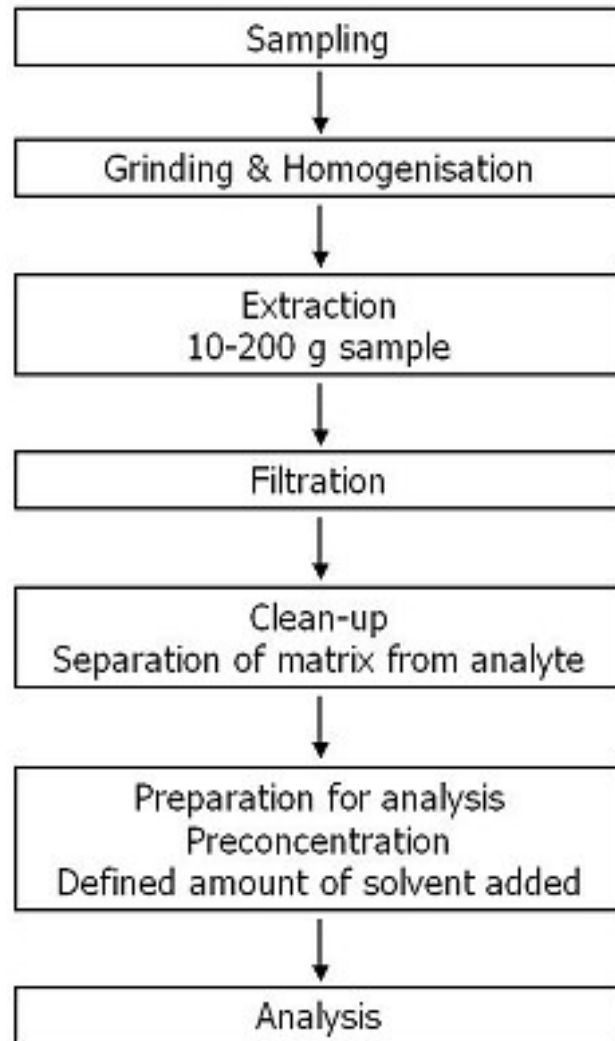
STACKING OF BAGS IN WARE-HOUSE



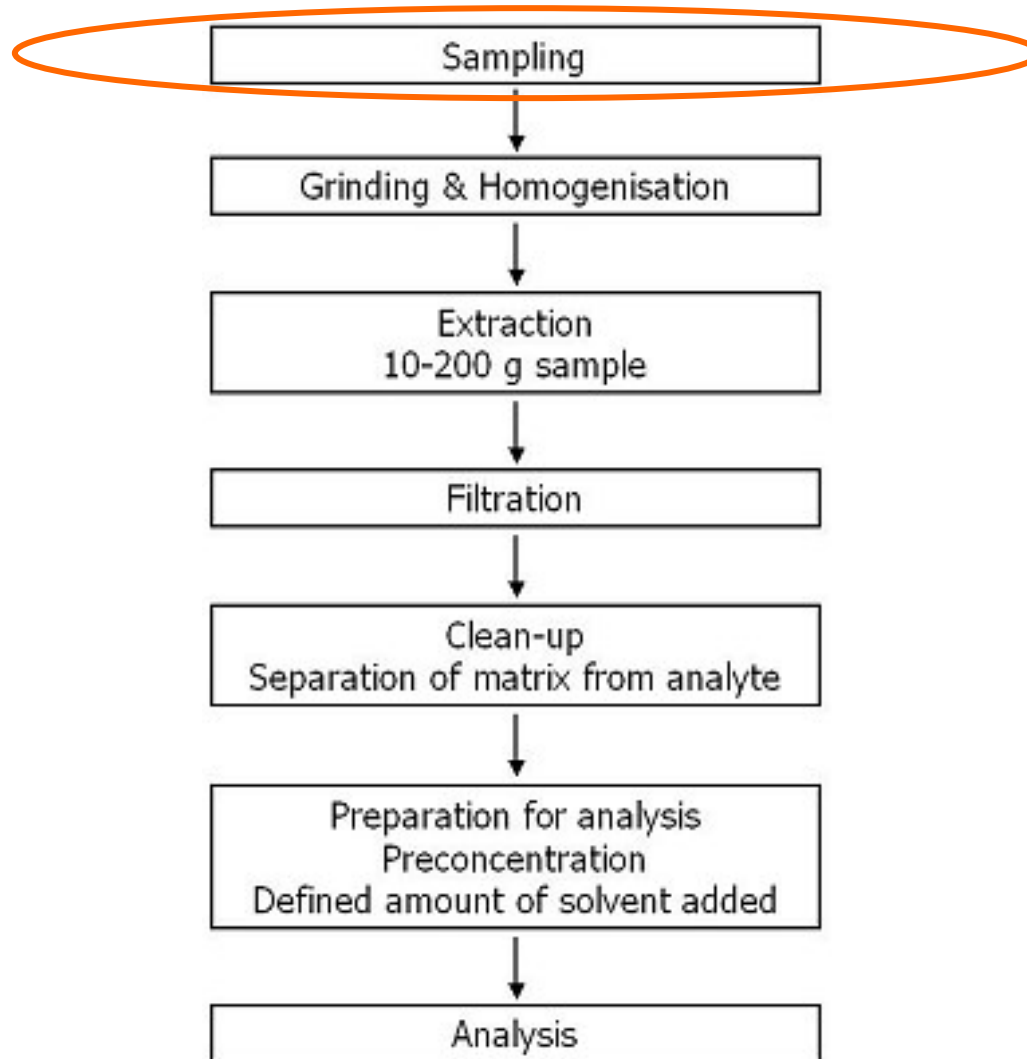
Correct Method

Incorrect Method

Mycotoxin Analysis - Main Steps



Mycotoxin Analysis - Main Steps



Sampling

- Major source of error and variation
- Mycotoxins are not evenly distributed in a lot
- Not every kernel or nut is contaminated
- A few kernels can contaminate large lots



Sampling

48	51	52
49	50	53
51	50	50
50	53	48

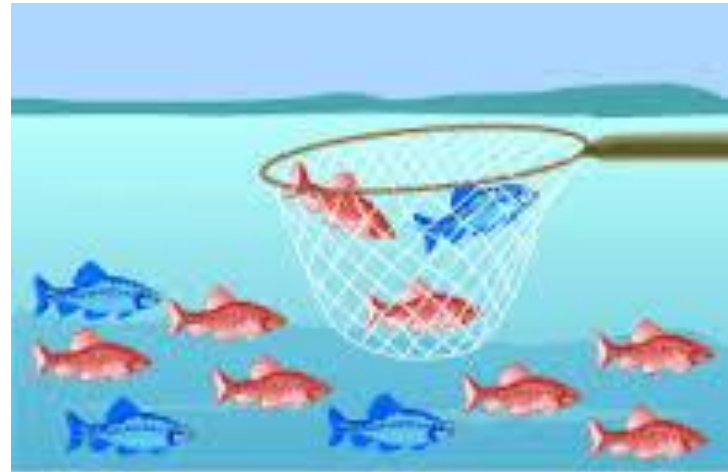
Protein

0	0	0
1	0	0
0	0	99
0	0	0

Aflatoxin

Sampling - Representative Sample

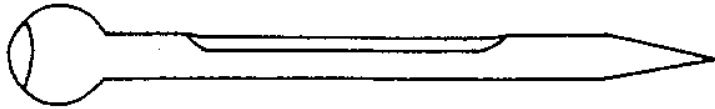
- In order for a sample to be representative it must:
 - Be obtained with equipment and procedures designed to obtain sample from all areas of the lot
 - Be of appropriate size
 - Be adequately identified
 - Be handled in such a way as to maintain its representativeness



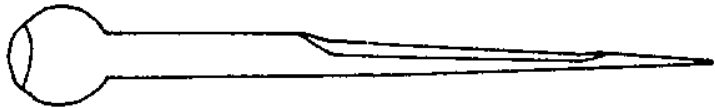
Sampling - Representative Sample

- Probes and bag triers

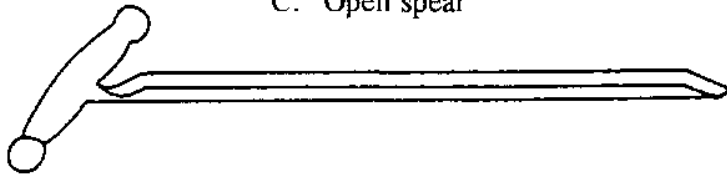
A: Closed spear for sampling large grains such as maize



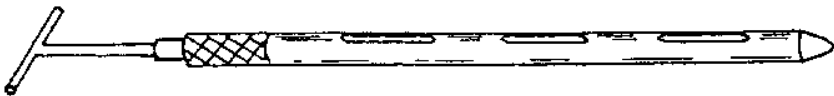
B: Closed spear for sampling small grains such as wheat



C: Open spear



D: Double-tube spear



Sampling - Representative Sample

- Sampling procedures



Sampling Devices

- Probes

- Standard lengths – 5, 6, 8, 10, and 12 feet
- The depth of the carrier defines the length of the probe used

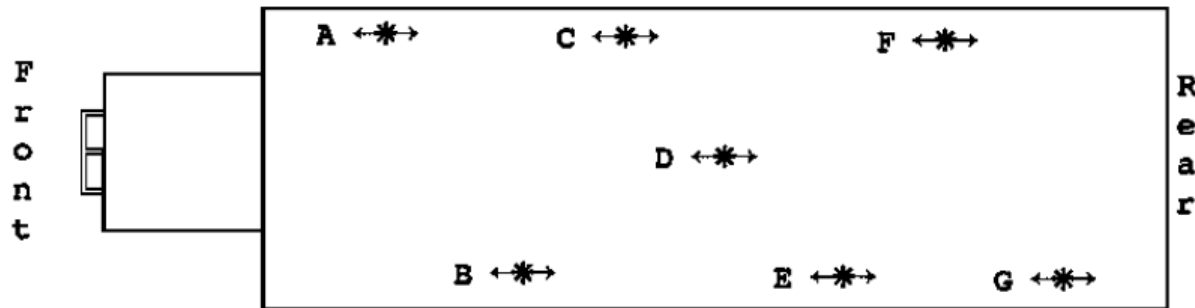


Carriers	Probe Lengths	Compartments
Barges and Bay Boats	12-foot	20 compartments
Hopper Cars	10- or 12-foot	20 compartments
Boxcars	6-foot	12 compartments
Trucks	5- or 6-foot	11 or 12 compartments
Hopper-Bottom Trucks	6-, 8-, or 10-foot	12, 16, or 20 compartments

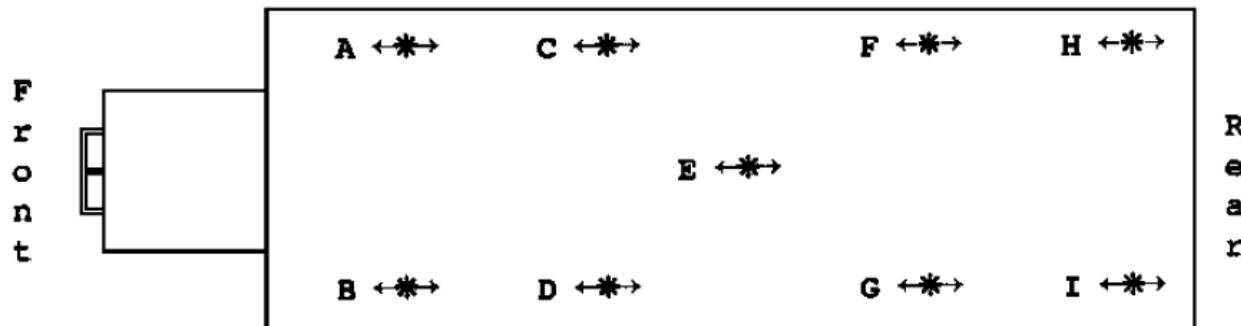
Other Containers - Use grain probes that will reach the bottom of the container.

Sampling Patterns

- Trucks - GIPSA
 - Flat bottom; grain more than 4 feet deep



- Flat bottom; grain less than 4 feet deep



Sampling Patterns

- Sacked grain - GIPSA
 - If the lot contains more than 10,000 sacks
 - ❖ Divide into 2 or more equal size sub-lots
 - ❖ From each sub-lot randomly select 36 sacks for sampling



Sampling - Sample Size

- According to GIPSA

Lot Type	Minimum Sample Size (lbs.)/ grams
Trucks	2 pounds / approximately 908 grams
Railcars	3 pounds / approximately 1,362 grams
Barges/Sublots	10 pounds / approximately 4,540 grams

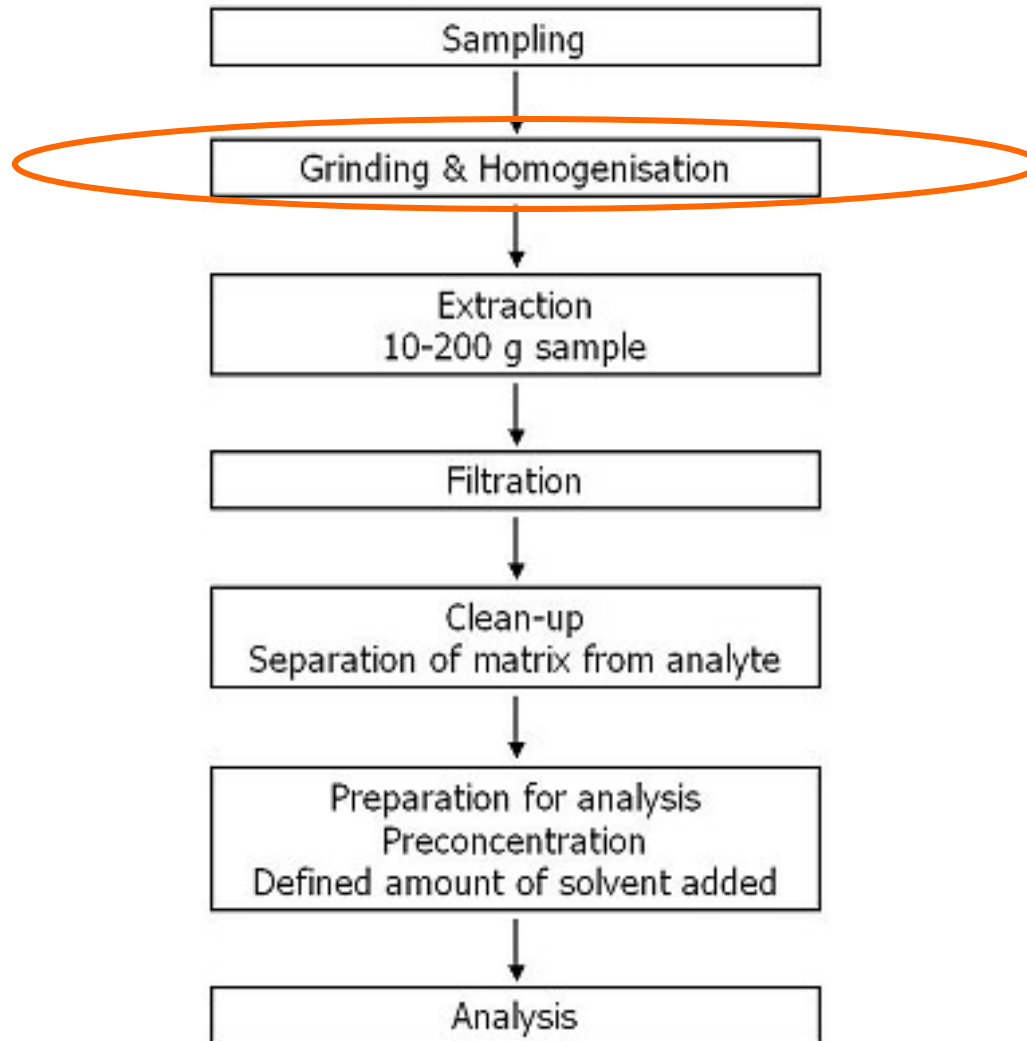
Truck Load = About 900 bushels of corn

Each corn bushel = 56 lbs

908 g sample = 0.04% of the load!

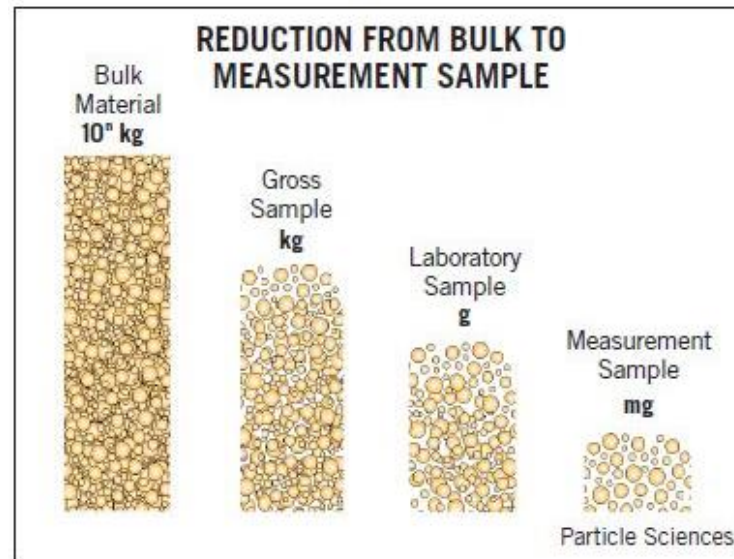
But never less than 1 kg!!

Mycotoxin Analysis - Main Steps

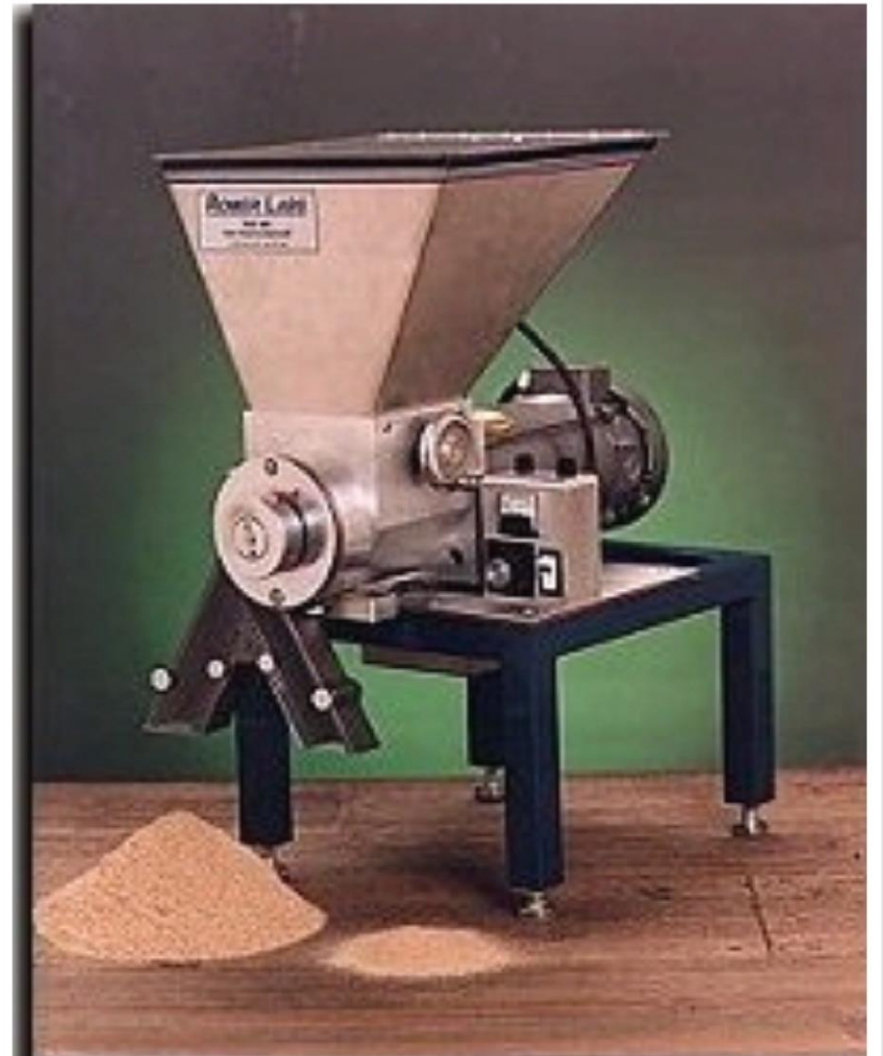


Sample Preparation - GIPSA

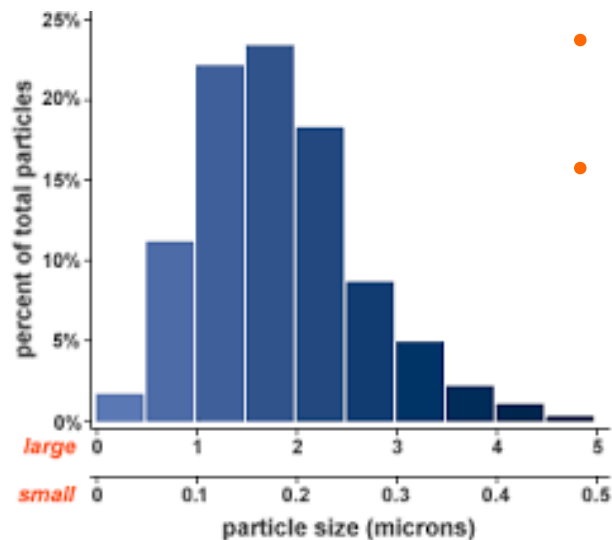
- Entire sample is ground in a mill
- Two 500 gram subsamples are taken
 - One for testing and another for retention
- From the 500 g work portion
 - Use a Boerner divide to remove 50 g for analysis



Sample Preparation - Size Reduction

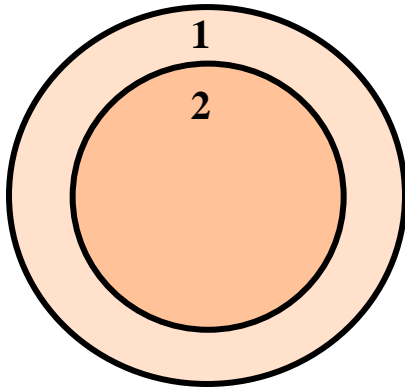


Size Reduction in the Field



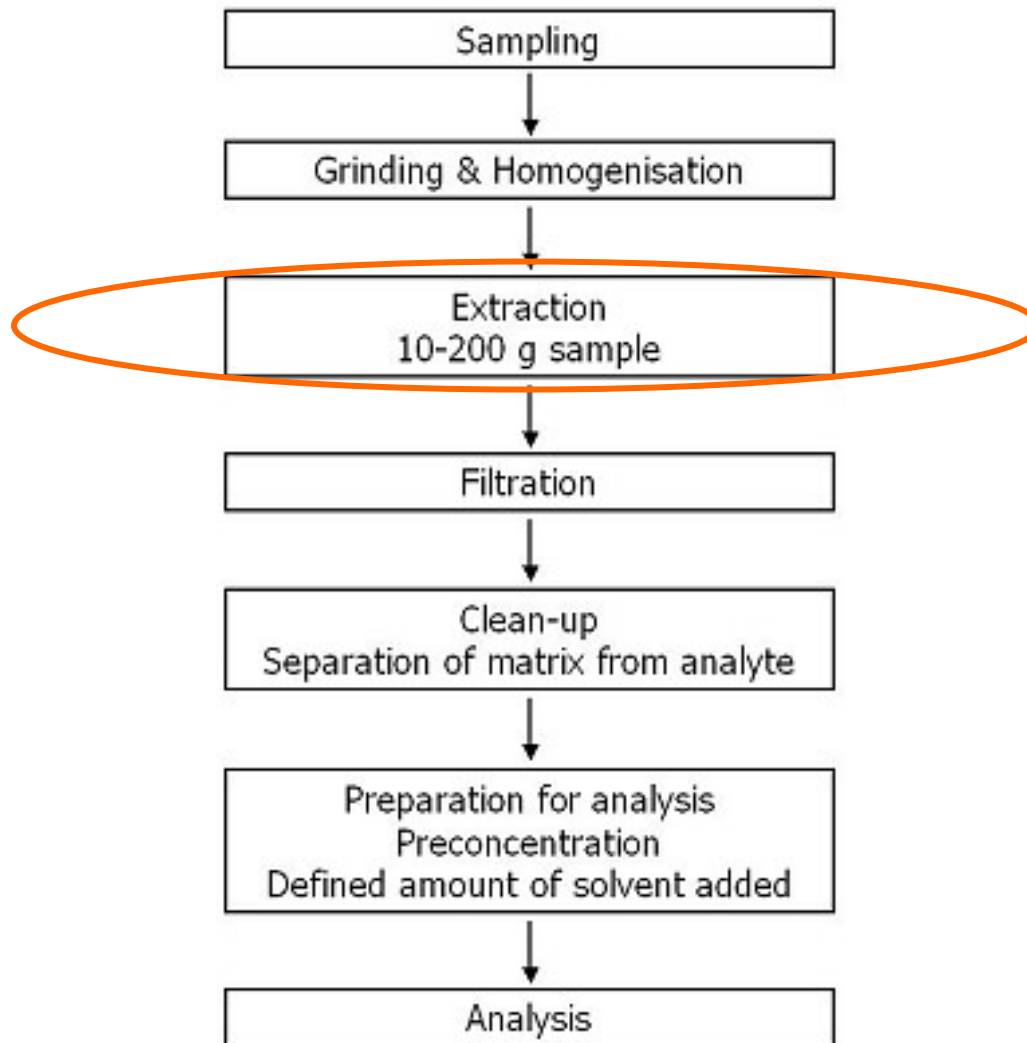
- The samples should be ground in its totality (300 g)
- According to Romer Labs:
 - Sample for analysis: 75% should pass through a 20-mesh screen (850 μm)
 - 5 min in the food processor: 52.56% of samples was smaller than 850 μm
 - 3 min in the ostar grinder: 81.22% of samples was smaller than 850 μm

Splitting Samples in the Field



- From original ground sample (1 kg):
 - 500 g: Mold and yeast counts
 - 500 g: Mycotoxin analysis
 - Remaining: Retain

Mycotoxin Analysis - Main Steps

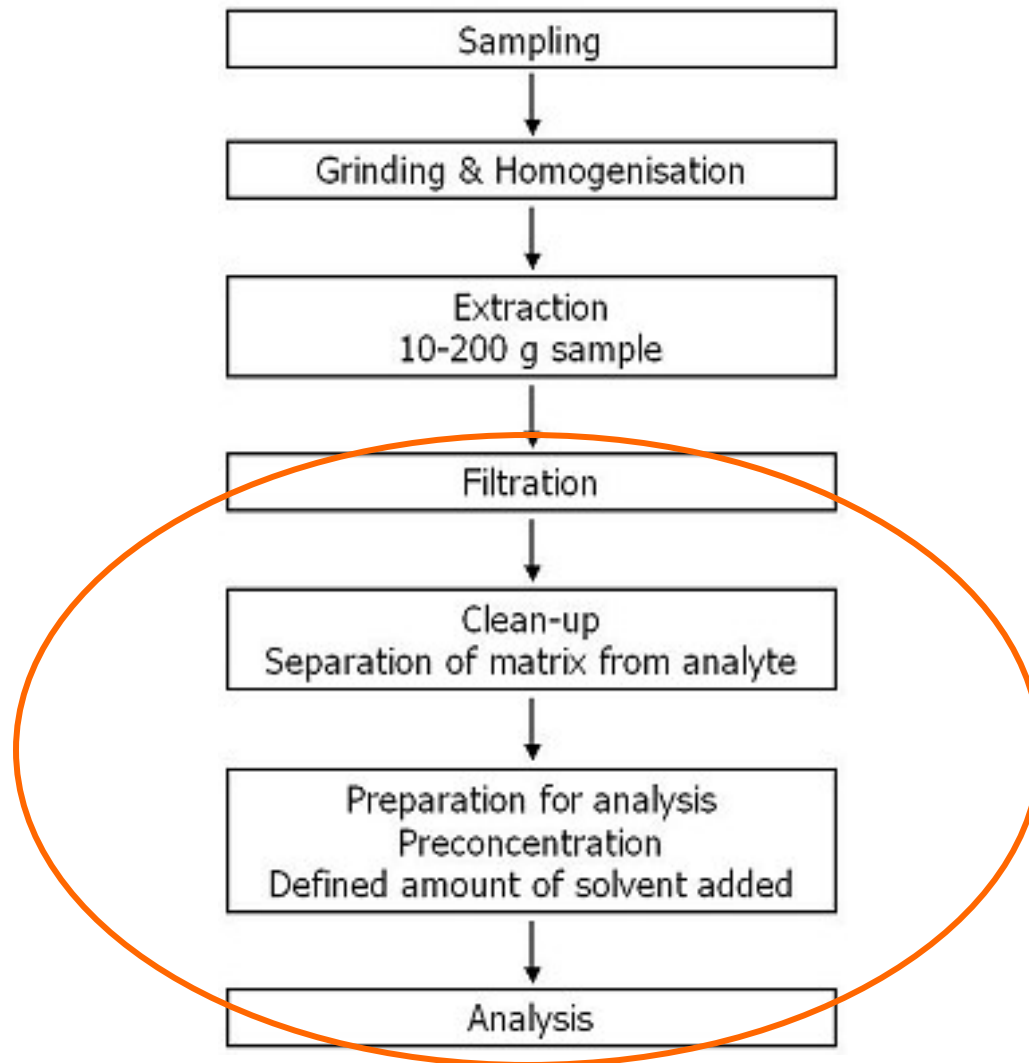


Mycotoxin Extraction

- Extraction solvent
 - Aqueous phase (phosphate buffer)
 - Organic solvent
 - Combination of both
 - ❖ Methanol and Water – Aflatoxins, Ochratoxin, Fumonisin
- Done in a blender or shaker



Mycotoxin Analysis - Main Steps



Analytical Procedures

- Officially approved methods should be used
 - Association of Official Analytical Chemists (AOAC)
 - USDA / GIPSA Approved
- Commercial test kits are available for
 - Aflatoxins
 - Deoxynivalenol
 - Ochratoxin A
 - T-2 Toxin



Analytical Procedures

- **Principles of the method**
 - Usually done in an antibody coated micro well plate or strip
 - Based on a competitive assay format
- **Advantages of test kits**
 - No need for clean-up
 - Fast
 - Cheaper than HPLC, CG
 - Equipment: microwell reader (visible light)
 - Some kits are approved by GIPSA for grain and grain based ingredients

Manufacturers/Suppliers of Test Kits

- Romer Laboratories (<http://www.romerlabs.com/>)
- Diagnostix (<http://www.diagnoxtix.ca/>)
- Neogen Corporation (<http://www.neogen.com/>)
- Strategic Diagnostics Inc (<http://www.sdix.com/>)
- VICAM (<http://www.vicam.com/>)
- R-Boppharm Rhone LTD (<http://www.r-biopharmrhone.com/>)
- R-Boppharm (<http://www.r-biopharm.com/>)

Romer - AgraStip

- One-step lateral flow immunochromatographic assay
 - Based on a competition immunoassay format
 - Antibody-particle complex (conjugate) lyophilized in a microwell
 - Sample is mixed with conjugate in microwell
 - Mixture is wicked onto a strip
 - In the strip the test zone captures free conjugate
 - ❖ The more color, the more toxin in the sample



Romer - AgraStip

Animation for ELISA - lateral flow



Mycotoxins in Guatemala

Type of corn Samples	Number of samples	Aflatoxin Levels (Average; Range)	Fumonisin Levels (Average; Range)
At harvesting	8	5.56ppb; <2ppb - 21.4ppb	0.15ppm; All samples <0.3ppm
After screening	4	3.65ppb; <2ppb - 6.34ppb	0.15ppm; All samples <0.3ppm
At beginning of storage (day 0)	9	4.31ppb; <2ppb - 8.86ppb	1.25ppm; <0.3ppm - 5.9ppm
Middle of storage (day 30)	1	3.78ppb	<0.3ppm

To this date only about 20% of the samples have being analyzed for mycotoxins.

Some values to keep in mind...

Type of Mycotoxin	Advisory Level (FDA)	Guidance Level (FDA)	Guidance/Regulatory Level (Others)
Aflatoxin	20 ppb		
Fumonisin		4 ppm (4,000 ppb) Corn for production of masa	
Deoxynivalenol		1 ppm (1,000 ppb) Finished wheat products 10 ppm (10,000 ppb) Raw grain	2 ppm (2,000 ppb) Raw grain CODEX (?)
Zearalenone			200 ppb Unprocessed corn/Corn for direct consumption 75 ppb Other cereal for direct consumption

Mycotoxins: An Overview

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KANSAS STATE
UNIVERSITY

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Lincoln

AFGHANISTAN MYCOTOXIN VALUE CHAIN ASSESSMENT PROJECT POST-HARVEST LOSS INNOVATION LAB

27 JULY, 2015



PURPOSE

- ▶ Undertake a rapid assessment of the prevalence of mycotoxins in the staple wheat food and high value horticulture value chains.
- ▶ The PHL Innovation Lab will provide the technical expertise to design and implement the assessment – with collaborative manner with MAIL.
- ▶ The PHL Innovation Lab will summarize findings, and propose recommendations for follow-up actions which should include ways to strengthen the institutional capacity of Afghan government and private sector entities to address mycotoxin contamination if it is identified as a problem.
- ▶ The assignment will be implemented under the USAID Bureau for Food Security's Leader with Associate mechanism for the Innovation Lab for the Reduction of Post-Harvest Loss (PHL Innovation Lab, the Awardee).

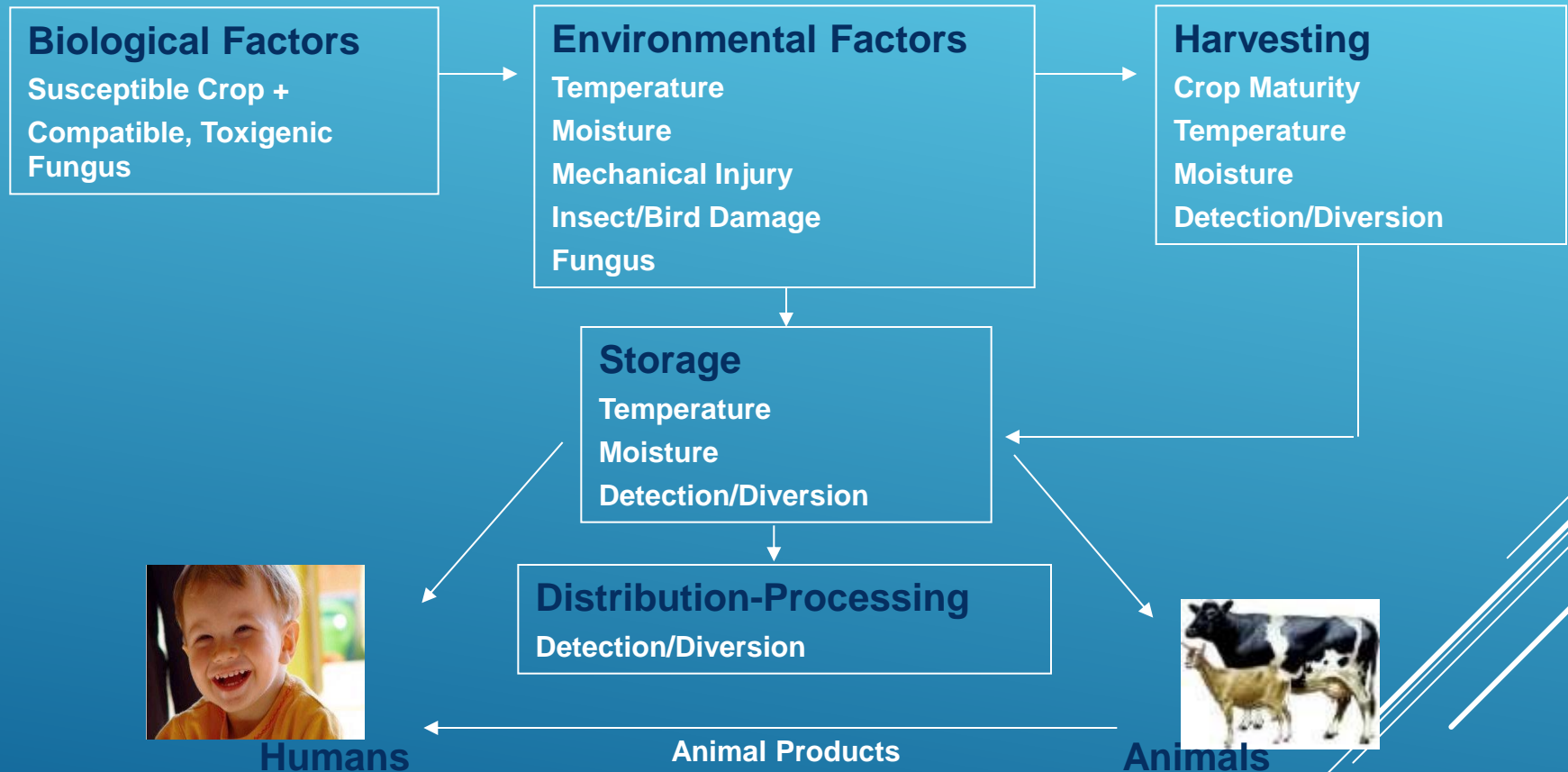
WHY MYCOTOXIN ASSESSMENT IS IMPORTANT?

- ▶ Mycotoxins are metabolites (by-products) of the growth of molds.
- ▶ Aflatoxins are naturally occurring mycotoxins that are produced by several *Aspergillus* species of fungi, the major ones are *Aspergillus flavus* and *Aspergillus parasiticus*.
- ▶ They have toxic side effects to plants, animals, and humans.
 - Aflatoxin B₁ is a potent liver carcinogen
 - Ochratoxin A and citrinin both affect kidney function.

WHO: Afghanistan ranks 6th in the world for liver cancer and 3rd for kidney cancer.

- ▶ Mycotoxin contamination of crops has been a worldwide problem for thousands of years.
- ▶ Only in the last thirty or forty years has technology allowed researchers to isolate the fungal mycotoxins and study the effects on feed crops, livestock, and their effects on humans. **3**

MYCOTOXIN ECONOMIC AND HEALTH RISKS



CONCERNS ABOUT MYCOTOXINS

- Where populations have a single dietary staple
 - May be exposed to great amounts
 - Acute and chronic toxicity possible
 - Less developed countries – more direct exposure



- Where diets are diverse
 - Low levels of exposure
 - Foods of better quality – lower amounts
 - More developed countries – direct and indirect exposure



- ❖ Food Ingredients
- ❖ Residues in animal products – milk, eggs, edible organ tissues

ADVERSE ECONOMIC EFFECTS OF MYCOTOXINS ON

LIVESTOCK

(Cows & poultry) and dairy

- ▶ Higher mortality rates
- ▶ Reproductive failures (abortions)
- ▶ Reduced feed efficiency
- ▶ Overall quality loss
- ▶ Lower milk production
- ▶ Nonmarketable milk

CROPS

- ▶ Yield Losses
- ▶ Restricted Markets
- ▶ Increased production costs
- ▶ Increased post harvest costs

PHL INNOVATION LAB IS EXPECTED TO ADDRESS AND UNDERTAKE THE FOLLOWING KEY ACTIVITIES

- 1. Design a research and sampling methodology**
- 2. Training of field staff in use of sampling technology**
- 3. Implementation of data collection**
- 4. Technical & material support to MAIL**
- 5. Assessment findings, draft report & presentation to MAIL, MoCI & MoPH**
- 6. International Workshop on pre and post-harvest loss reduction**

METHODOLOGY

The PHL Innovation Lab will work with MAIL staff throughout the project area to collect required information, analyze and compile data. This will be done through,

- ▶ **Document Review**
- ▶ **Consultation with MAIL and other GIRoA ministries**
- ▶ **Design of Research approach**
- ▶ **Field sampling:**
- ▶ **Information Collection and Analysis:**
- ▶ **Major findings and/or important next steps with MAIL and USAID**

FUSARIUM TRICINCTUM (T2)

- In humans, it has been linked to:
 - Esophageal cancer in South Africa, Northeast Italy and Northern China
 - Neural tube defects in developing human embryos
 - The most dangerous of the mycotoxins
- Mainly found in wheat



SAMPLING

- Major source of error and variation
- Mycotoxins are not evenly distributed in a lot
- Not every kernel or nut is contaminated
- A few kernels can contaminate large lots



SAMPLING

48	51	52
49	50	53
51	50	50
50	53	48

Protein

0	0	0
1	0	0
0	0	99
0	0	0

Aflatoxin

DELIVERABLES

- ▶ Detailed Methodology and Action Plan
 - ▶ Progress Update
 - ▶ Final Report
 - ▶ Stakeholder Briefings
 - ▶ MAIL Lab support
 - ▶ MAIL training
 - ▶ International Workshop in 2015
- ▶ USAID will circulate the results of this investigation among development partners with the intent of prompting further research and appropriate measures to improve food quality and reduce post-harvest losses. The Awardee will organize an international workshop on post-harvest losses and food quality towards the end of this project where the results and mitigation recommendations will be presented. This workshop will take place outside of Afghanistan in the summer of 2015.



KANSAS STATE
UNIVERSITY

UNIVERSITY OF
Nebraska
Lincoln

THANK YOU



PHL Innovation Lab Afghanistan

Sampling Procedure Protocol

27 July 2015



KANSAS STATE
UNIVERSITY

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Nebraska
Lincoln



OUTLINE

- Types of sampling
- Sampling Procedure Protocol
- Sample collection
- Sampling tools
- Packing and labeling of samples
- Submission sample for Analysis

Types of Sampling

1. **Primary sample:** Each probe or handful of sample taken either in bag or in bulk is called primary sample.
1. **Composite sample:** All the primary samples drawn are combined together in suitable container to form a composite sample.
1. **Submitted sample:** When the composite sample is properly reduced to the required size that to be submitted to the Wheat, Raisin and Nuts testing laboratory, it is called submitted sample.
1. **Working sample:** It is the reduced sample with required weight obtained from the submitted sample after repeated mixing and dividing with which the seed quality tests are conducted in Wheat, Raisin and Nuts testing laboratory.

Sampling Procedure Protocol



Methods and Types of Sampling

Objectives:

- Sampling is done to get a uniform and representative sample from a wheat/dry fruits lot. The size of the submitted sample required for testing is small as compared to the size of the lot, therefore, care must be taken to ensure that the submitted sample represents the lot of the wheat/dry fruits to be tested.
- Hence it is essential that the samples be prepared in accordance to following guidelines (sampling protocol) to ensure that the small size sample should represent truly and in the same proportion all constituents of seed lot.

Wheat/Wheat flour



Sampling Continue.....

To describe how a sample has to be taken, to be representative of a specific lot.

What is a lot?

- The total amount of flour obtained after grinding what a farmer brought in to the Asiab mill;
- The total amount of flour produced in a day or half-day in a commercial mill;
- The total amount of flour or wheat a farmer have stored in their house (it could be a single bag or several bags);
- The total number of bags a vendor may have available at the market coming from a single source (i.e. received all at once from a specific region of Afghanistan or imported from a specific country) or the total number of bags a vendor may have of a specific type of flour/wheat.
- The total number of flour/wheat bags in a warehouse from a single source (i.e. received all at once from a specific region of Afghanistan or imported from a specific country);
- The total number of flour/wheat bags a warehouse may be storing, if there is no information that help further segregate those bags. An example of further segregation would be flour/wheat received from a single source or a specific harvesting year;

Raisins and Nuts



Raisins and Nuts

To describe how a sample has to be taken, to be representative of a specific lot.

What is a lot?

- The total amount of raisins or nuts drying at a small processor;
- The total amount of raisins or nuts processed in a day or half-day in a commercial facility;
- The total amount of raisins or nuts a farmer have stored in their house (it could be a single bag or several bags);
- The total number of bags a vendor may have available at the market coming from a single source (i.e. received all at once from a specific region of Afghanistan or imported from a specific country) or the total number of bags a vendor may have of a specific type of raisins or nuts (i.e. pistachio shelled or unshelled, walnuts, paper shell almonds or other variety).
- The total number of bags of raisins or nuts in a warehouse from a single source (i.e. received all at once from a specific region of Afghanistan or imported from a specific country) or type (i.e. dark raisins or yellow raisins, paper shell almonds or other types);
- The total number of bags of raisins or nuts a warehouse may be storing, if there is no information that help further segregate those bags. An example of further segregation would be product received from a single source, of a single variety or a specific harvesting year;



SAMPLING PROCEDURES

For bagged or piled products (i.e. flour, grain, nuts, raisins)

In order to obtain a representative sample from a lot a sufficient number of sub-samples must be taken using the following rules:

- a. If there is only one bag/pile of product, randomly select at least 5 sampling points and take a sub-sample from each point.
- b. If there are up to 10 bags of product, take one sub-sample per bag.
- c. If there are between 11 to 100 bags of product, randomly select 10 bags and take one sub-sample per bag.

Note: In the case of Asiab mills, sub-samples should be taken at intervals during grinding (i.e. beginning of grinding of a wheat lot, middle of grinding and at the end of the process).



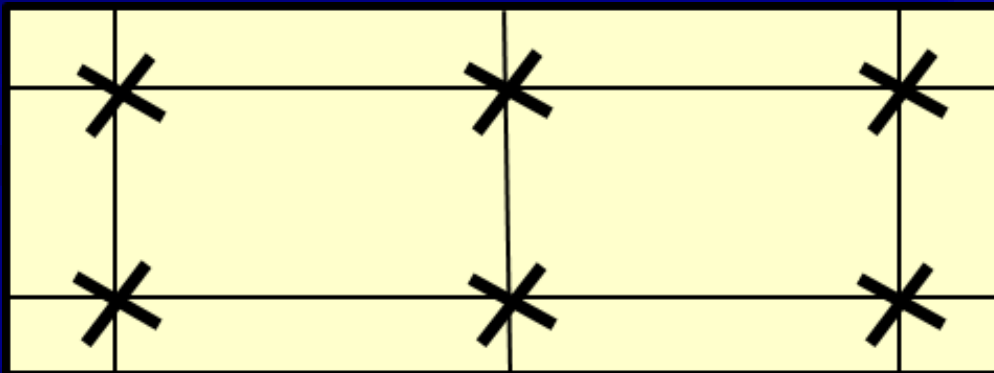




Sampling points

The sampling points must be evenly distributed over the total lot/pile surface according to a grid sampling pattern (figure 1). If samples are in bags, using a sampler, take sub-samples at regularly spaced intervals over a given space (lot). Choose an initial location at random, and then define the remaining sampling locations so that all locations are at regular intervals over an area; for example, at the points identified by the intersection of each line in the grid shown in figure 1. If samples are piled, and the use of the probe is not possible due to a low height of the material, then follow the same grid pattern but use a measuring cup to obtain the sub-samples.

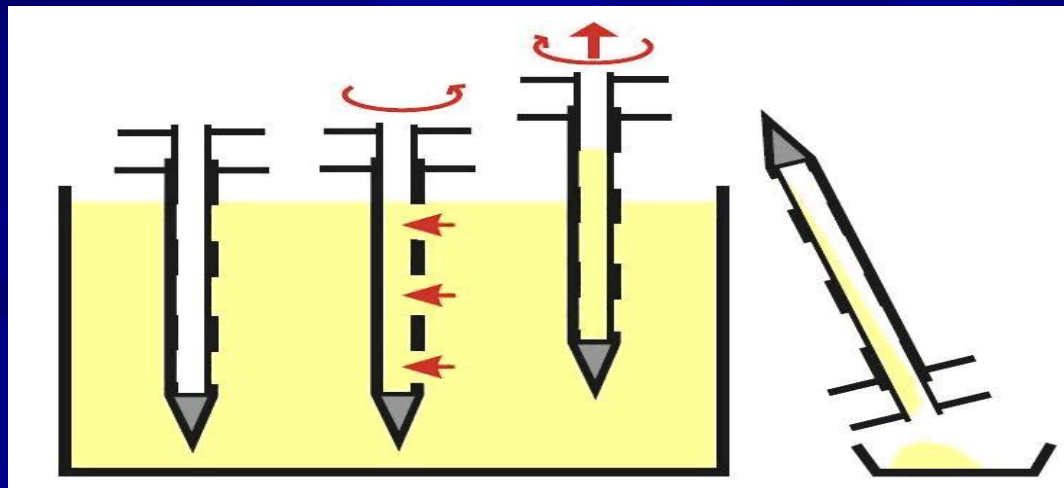
Grid Pattern



Using probe sampler:

follow the steps listed from a through d (see figure 2):

- Insert the sampler into the product bag/container (A)
- Rotate the inner tube through 180° (B), to open the sampler. The product can now flow into the slot sampler.
- Rotate the inner tube through 180° to close the sampler and withdraw the sampler (C).
- Pull out the inner tube and deposit the sample into a plastic container (D).



Using a sampler

For product spread out or hanging for drying (i.e. nuts and raisins)

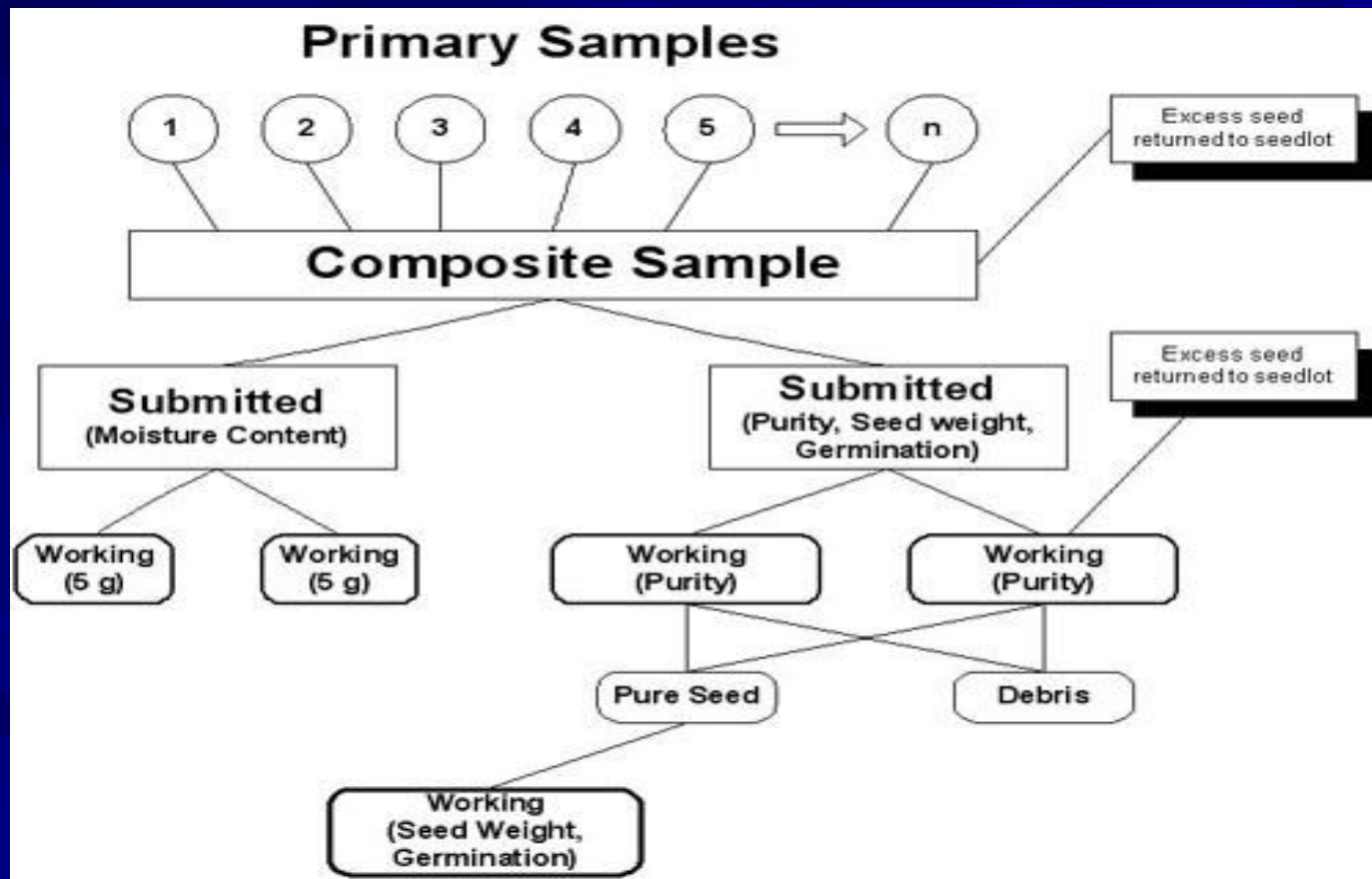
In order to obtain a representative sample from a lot a sufficient number of sub-samples must be taken using the following rule:

From the area where the product is drying randomly select at least 5 sampling points and take a sub-sample from each point. Figure 1 could be used as illustration of points for sampling almonds that may be spread out on the floor for drying or raisins that may be hanging from a wall.

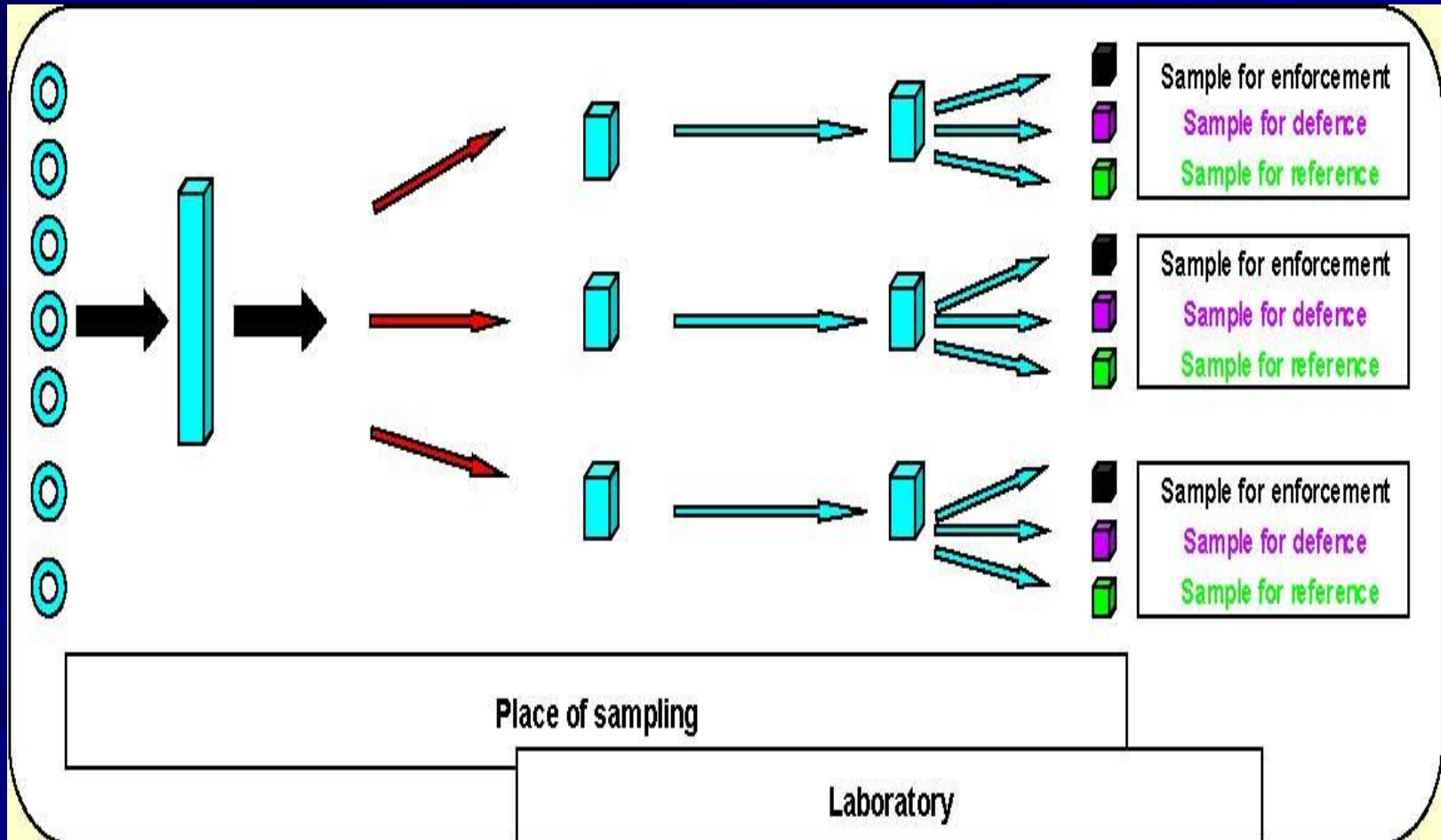


Take Composite Sample

Once all the sub-samples have been collected from the selected sampling points, thoroughly mix the sub-samples into a plastic container (tub or bucket) to obtain a composite sample.



Samples for testing, defense and reference taken from composite or sub samples.



From the composite sample, take the following number of sub-samples using a 1-liter plastic cup to obtain at least 500 g of shelled product:

- a) Wheat flour: 1 full cup
- b) Almonds without shell: 1 full cup
- c) Almonds with shell: 3 full cups
- d) Walnuts without shell: 2 full cups
- e) Raisins: 1 full cup
- f) Pistachios without shell: 1 full cup
- g) Pistachio with shell: 2 full cups

Labeling

USAID **KANSAS STATE UNIVERSITY** **Nebraska**

Feed the Future Innovation Lab for the Reduction of Post-Harvest Loss

Individual sample information

Sample description: Wheat Maize Rains

Date of sample collection:
MM DD YY

Days sample has been stored (since production/harvest, if known):

Type of storage:

Sample origin:

Location:
(of sample provider)

Produced: Bought Other

For "Other" please specify: _____

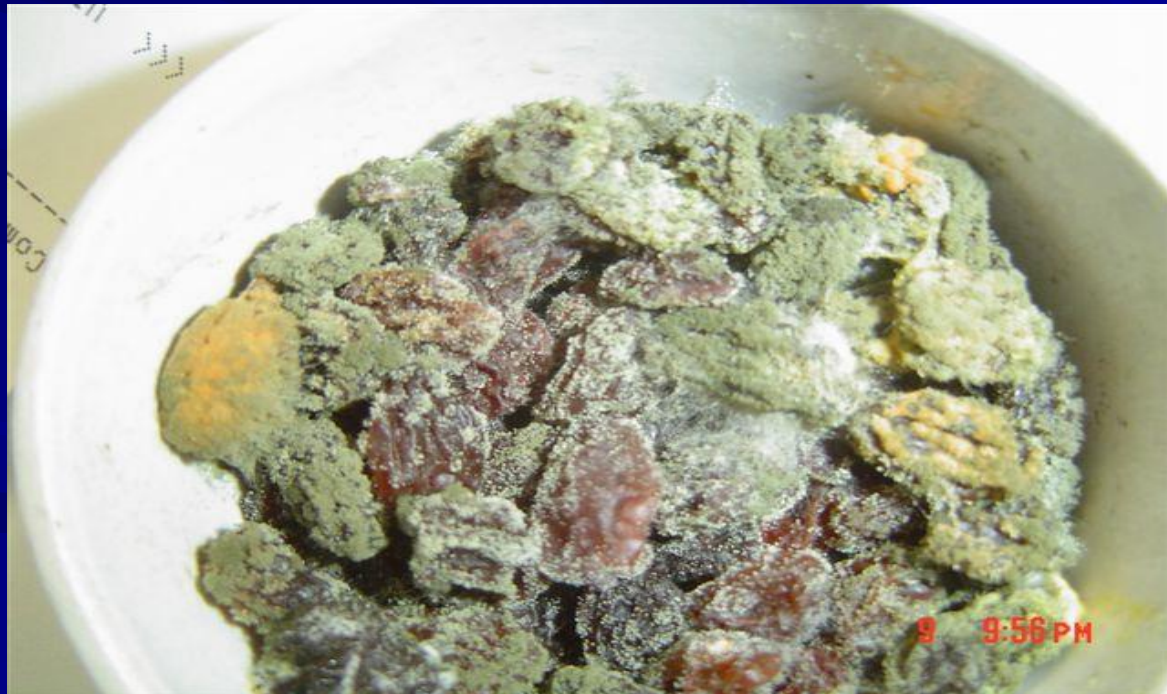
- Place the 500 g sample in sterile plastics bags, properly labeled
- The plastic cups must be cleaned after the sample is placed in the sterile plastic bag using wet wipes to remove any dust/particles adhered to the walls, followed by drying using paper towels.

Important Note:

- Transfer samples to the laboratory in Kabul as soon as possible. In the meantime, store the samples in a clean dry place, away from pests such as insects, rodents or birds, until they are ready for shipment and further analysis. If extended storage (i.e., more than 3 days) is needed before sample is transferred to laboratory then samples should be frozen.
- If samples are not shipped when expected, they must be stored in the freezer until the next day of shipping.
- Sample information must be added to “Sampling control log (AFG)” prior to shipment.

THANKS
ANY QUESTIONS??

سوالات؟









Proposed Wheat / Flour / Wheat Products Sampling

	Type of Samples	Number of Samples
W01	Asiabs Mill Flour of Afghan Origin	103 - 156
W02	Grist Mill Flour of Afghan Origin	
W03	Asiabs and Grist Mill Flour of Kazakhstan Origin	5 - 10
W04	Asiabs and Grist Mill Flour of Uzbekistan Origin	3 - 7
W05	Purdue Improved Crop Storage (PICS) bags	4
W06	Two warehouses or storage facilities in Mazar-i-Sharif, Kabul, and Herat, as recommended by grain traders or farmers.	6
W07	Two naan bakeries in each of the three regions	9 - 22
W08	Two – four flour millers in each of the three regions	9 - 22
W09	In Kabul market sampling of Pakistan flour	6 - 12
W10	In Herat market sampling of Iran wheat products	3 - 6
W11	In Kabul market sampling of other flour	2 - 5
	TOTAL	150 - 250

Proposed Raisins Sampling Scheme.

	Type of Samples	Number of Samples
R01	Medium Quality Round Green Raisin	17 - 29
R02	Medium Quality Long Green Seedless Raisin	17 - 28
R03	High Quality Shundurkhani Raisin (Golden-High Value)	17 - 29
R04	Medium Quality Red Raisin	17 - 28
R05	Sun dried Shomali Raisin	17 - 29
R06	Sun dried Ghazni Raisin	17 - 28
R07	Sun dried Tayefe Raisin (Mazar-i-Sharif)	18 - 29
R08		
R09	Other OR Mixed Raisin	
	TOTAL	120 - 200

Proposed Almond Sampling Scheme.

	Type of Samples	Number of Samples
A01	Sattarbai Soft-shell Almonds (Mazar-i-Sharif)	15 - 25
A02	Shokorbai Hard-shell Almonds	15 - 25
A03	Abdul Wahidi Almonds (Mazar-i-Sharif)	15 - 25
A04	Qambari Almonds	15 - 25
A05	Ghorbandi Almonds	15 - 25
A06	Sangaki and Murawaji Almonds (smaller kernels)	15 - 25
A07	OTHER ALMOND	
	TOTAL	90 - 150

Proposed Pistachio Sampling Scheme.

	Type of Samples	Number of Samples
P01	Korak Pistachios	23 - 38
P02	Pushdara Pistachios	23 - 38
P03	Khandan-e-safid Pistachios	23 - 38
P04	Other varieties of Pistachios	21 - 36
	TOTAL	90 - 150

Proposed Walnut Sampling Scheme.

	Type of Samples	Number of Samples
WN01	Zard Walnuts (yellow kernels)	10 - 17
WN02	Mazaari Walnuts	10 - 17
WN03	Takhari Walnuts	10 - 17
WN04	Korek Walnuts	10 - 17
WN05	Kaghazi Walnuts (paper shelled)	10 - 17
WN06	Other varieties of Walnuts	10 - 15
	TOTAL	60 - 100

Appendix IV – Manufacturer ELISA test kit protocols

IV.1 Romer AgraQuant[®] Total Aflatoxin Assay 4/40

IV.2 Romer AgraQuant[®] Ochratoxin Assay 2/40

IV.3 Romer AgraQuant[®] T-2/HT-2 Toxin Assay 25/500

IV.4 Romer AgraStrip[®] Deoxynivalenol (DON) Quantitative Test



AgraQuant[®] Total Aflatoxin
Assay 4/40

Order No.:
COKAQ1000/COKAQ1048

Romer Labs Singapore Pte. Ltd.
Tel: (65) 6631 8018
Fax: (65) 6275 5584
Web: <http://www.romerlabs.com>

This Package Insert is available in following languages:

- Spanish
- French
- Portuguese
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Email: salesasia@romerlabs.com



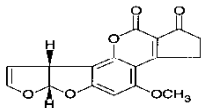
AgraQuant[®] Total Aflatoxin Assay 4/40 Competitive ELISA



Order #: COKAQ1000/COKAQ1048

Aflatoxins

Aflatoxins are toxic and carcinogenic. They are metabolites of the fungi *Aspergillus flavus* and *Aspergillus parasiticus*. There are four principle types of aflatoxin: B₁, B₂, G₁ and G₂, which are named for their respective innate fluorescent properties. Aflatoxin B₁ is the most frequently encountered of the group and the most toxic. Aflatoxins can be found mainly in cereals, corn, peanuts, cottonseed and nuts.



Aflatoxin B1

Short Instruction:



1 Pipette 200 μ L conjugate solution into dilution wells

2 Add 100 μ L of each standard or sample extract into the dilution wells.

3 Mix well and transfer 100 μ L from dilution wells into antibody coated wells, incubate at RT for 15 minutes

4 Wash 5 times with distilled/deionized water

5 Tap dry washed wells

6 Pipette 100 μ L substrate solution into the antibody coated wells, incubate at RT for 5 minutes

7 Pipette 100 μ L stop solution into the antibody coated wells

8 Read the strips with ELISA reader using 450nm filter and 630nm differential filter



Performance Characteristics:

<u>LOD:</u>	3 ppb for corn and other commodities 5 ppb for Sorghum 6 ppb for DDGS
<u>LOQ:</u>	4 ppb
<u>Range:</u>	4-40 ppb

Sample Preparation / Extraction

1. Obtain a representative sample and grind it using a Romer Series II® Mill so that 75% will pass through a 20-mesh screen, then thoroughly mix the subsample portion.
2. Weigh out 20 g of ground sample into a clean jar that can be tightly sealed. (For corn bran, weight out 10g instead of 20g).
3. Add 100 mL of 70/30 (v/v) methanol/water extraction solution and seal jar. Note: Samples (except corn bran) should be extracted in a ratio of 1:5 (w:v) of sample to extraction solution respectively. (For corn bran, add 100mL of 70/30 (v/v) methanol/water extraction solution to 10g of ground sample and seal jar; the extraction ratio is 1:10 (w/v). The final result of aflatoxin in corn bran is the ELISA testing result times the dilution factor of 2).
4. Vigorously shake or blend for 3 minutes.
5. Allow sample to settle, then filter the top layer of extract through a Whatman #1 filter and collect the filtrate. Note: Commodity extracts should have a pH of 6-8. Excessive alkaline or acidic conditions may affect the test results and should be adjusted before testing.
6. Sample is now ready for testing. (Except for walnuts and mooncake, their extracts need to be cleaned with a



Mycosep 112 column before testing, contact technical service for details).

Assay Procedure in Detail

Note: All reagents and kit components must be at room temperature 18-30°C (64-86°F) before use. It is recommended that an 8-channel pipettor be used to perform the assay. No more than 48 samples and standards total (6 test strips) should be run in one experiment when using an 8-channel pipettor. If an 8-channel pipettor is not used (i.e. using only single channel pipettes), it is recommended that no more than a total of 16 samples and standards (2 test strips) be run in any one experiment.

1. Place the appropriate number of blue/green-bordered Dilution Strips in a microwell strip holder. One Dilution Well will be required for each standard, (i.e. 0, 4, 10, 20, & 40 ppb) or sample.
2. Place an equal number of Antibody Coated Microwell strips in a microwell strip holder. Return unused microwell strips to the foil pouch with the desiccant packet and reseal pouch with tape.
3. Measure the required amount of Conjugate from the green-capped bottle (~240 μL /well or 2 mL/strip) and place in a separate container (e.g. reagent boat when using the 8-channel pipettor). Using an 8-channel pipette, dispense 200 μL of Conjugate into each blue/green-bordered Dilution Well.
4. Using a single channel pipettor, add 100 μL of each standard or sample into the appropriate Dilution Well containing 200 μL of Conjugate. Use a fresh pipette tip for



each standard or sample. Note: Make sure the pipette tip has been completely emptied.

Using an 8-channel pipettor with fresh tips for each 8-well strip, mix each well by carefully pipetting it up and down 3 times and immediately transfer 100 μL of the contents from each Dilution Well into a corresponding Antibody Coated Microwell. Incubate at room temperature for 15 minutes. Note: Do not agitate the plate to mix as it may cause well-to-well contamination.

5. Empty the contents of the microwell strips into a waste container. Wash by filling each microwell with distilled or deionized water, and then dumping the water from the microwell strips. Repeat this step 4 times for a total of 5 washes. Note: Take care not to dislodge the strips from the holder during the wash procedure.
6. Lay several layers of absorbent paper towels on a flat surface and tap microwell strips on towels to expel as much residual water as possible after the fifth wash. Dry the bottom of the microwells with a dry cloth or towel.
7. Measure the required amount of Substrate from the blue-capped bottle ($\sim 120 \mu\text{L}/\text{well}$ or 1 mL/strip) and dispense into a separate container (e.g. reagent boat for an 8-channel pipettor). Pipette 100 μL of the Substrate into each microwell strip using an 8-channel pipettor. Incubate at room temperature for 5 minutes.
8. Measure the required amount of Stop Solution from the red-capped bottle ($\sim 120 \mu\text{L}/\text{well}$ or 1 mL/strip) and dispense into a separate container (e.g. reagent boat for an 8-channel pipettor). Pipette 100 μL of Stop Solution into each microwell strip using an 8-channel pipettor. The color should change from blue to yellow.
9. Read the strips with a microwell reader using a 450 nm filter with a 630nm differential filter. Record OD readings



for each microwell. Note: Air bubbles should be eliminated prior to reading strips as they may affect analytical results.

Additional Notes: Ratio of Conjugate to Standard/Sample should remain at 2:1, but volumes of Conjugate and Standards/Samples can be reduced, e.g. using 100 μ L and 50 μ L, respectively. The content to be transferred from dilution well to antibody coated well remains the same as 100 μ L. Do not return unused reagents to their original bottles. Carefully keep track of the position of Samples and Standards during the assay. Do not mix the assay microwells by shaking at any time during test.

Interpretation of Results

Using either the unmodified OD values or the OD values expressed as a percentage of the OD of the zero (0) standard, construct a dose-response curve using the five standards. Since the amount of aflatoxin in each standard is known, the unknowns can be measured by interpolation from this standard curve. Results can also be easily calculated using the Romer® Log/Logit spreadsheet that is provided (free of charge) upon request. If the Log/Logit regression model is used for results interpretation, the linearity coefficient (r^2) of the calibration curve should be no less than 0.985. An OD value of less than 0.5 absorbance units for 0ppb standard may indicate deterioration of reagents.

If a sample contains aflatoxin levels higher than the highest standard (>40 ppb), the filtered extract should be further diluted in 70% methanol such that the diluted sample results are in a range of 5 - 20 ppb and reanalyzed to obtain accurate results. The dilution factor must be included when the final result is calculated.



Performance Characteristics in Detail

Limit of detection:

3 ppb for corn and other commodities (except for Sorghum which is 5ppb and for DDGS which is 6ppb) (Determined by the average values of 10 aflatoxin-free samples plus 2 standard deviation).

Limit of quantitation:

4 ppb (Described as the lowest concentration point on the calibration curve that this test can reliably detect aflatoxin).

Range of quantitation:

4 – 40 ppb (For quantitation of samples above 40 ppb samples should be diluted such that the diluted sample result are in a range of 5 - 20 ppb; the test kit has been validated for assaying sample concentrations up to 320ppb).

Note: For corn germ meal and corn gluten feed limits of detection and quantitation, contact technical services.



Materials supplied

Order #: COKAQ1000

- 96 antibody coated microwells (12 eight-well strips) in a microwell holder (sealed in a foil pouch)
- 96 non-coated dilution microwells (12 eight-well strips marked with blue/green at base)
- 5 vials of 1.5mL of each aflatoxin standard (0, 4, 10, 20 and 40 ppb)
- 1 bottle of 25mL of aflatoxin conjugate (green-capped bottle)
- 1 bottle of 15mL of substrate solution (blue-capped bottle)
- 1 bottle of 15mL of stop solution (red-capped bottle)

Order #: COKAQ1048

- 48 antibody coated microwells (6 eight-well strips) in a microwell holder (sealed in a foil pouch)
- 48 non-coated dilution microwells (6 eight-well strips marked with blue/green at base)
- 5 vials of 0.75mL of each aflatoxin standard (0, 4, 10, 20 and 40 ppb)
- 1 bottle of 12.5mL of aflatoxin conjugate (green-capped bottle)
- 1 bottle of 7.5mL of substrate solution (blue-capped bottle)
- 1 bottle of 7.5mL of stop solution (red-capped bottle)

Materials required but not supplied

Extraction Procedure

- *EQMMS2010: Romer Series II[®] Mill or equivalent
- *EQOLE1025: Blender or a tightly sealing jar with lid
- *EQOLE1010: Balance, 400 g



- *EQOLE1050: Graduated cylinder: 100mL
- *70% methanol or
 - ACS grade methanol for making 70 % methanol
 - Distilled or de-ionized water for making 70 % methanol
- Container with a minimum 125mL capacity
- *Whatman#1 filter paper, or equivalent
- *Filter funnel
- *MycoSep112 column

Assay Procedure

- *8-channel and single channel pipettors capable of pipetting 100 μ L and 200 μ L with tips
- *EQOLE1300: Timer
- *COKAD1150: Wash bottle
- Distilled or de-ionized water
- Absorbent paper towels
- *3 reagent boats for use as reagent containers for an 8-channel pipettor
- *Microwell reader with a 450nm filter and a 630nm differential filter or equivalent.

*Items available from Romer Labs, Inc.[®] - Americas Division

Technical and Background Information

The AgraQuant[®] Total Aflatoxin Assay is a direct competitive enzyme-linked immunosorbent assay (ELISA) that determines a quantitative level for the presence of total aflatoxin (B₁, B₂, G₁ and G₂) and is intended for use in grains, cereals, nuts, animal feeds and other commodities.

The AgraQuant[®] Total Aflatoxin Assay has been validated for almond, corn, corn meal, corn gluten meal, corn bran, corn/soy blend,



cottonseed, dried distillers grains (DDGS), milled rice, mooncake, peanuts, popcorn, sorghum, soybeans, walnut and wheat.

Aflatoxins

Aflatoxins are toxic and carcinogenic. They are metabolites of the fungi *Aspergillus flavus* and *Aspergillus parasiticus*. There are four principle types of aflatoxin: B₁, B₂, G₁ and G₂, which are named for their respective innate fluorescent properties. Aflatoxin B₁ is the most frequently encountered of the group and the most toxic. Aflatoxins can be found mainly in cereals, corn, peanuts, cottonseed and nuts.

Aflatoxins can cause liver disease in animals and may cause decreased production (milk, eggs, animal weight, etc). Aflatoxin B₁ is a potent human carcinogen, and may contribute to human liver cancer.

The US Food and Drug Administration action levels of aflatoxin are as follows: (1) 300ppb for feeder cattle; (2) 200ppb for finishing swine; (3) 100ppb for breeding beef cattle, swine and mature poultry; and (4) 20ppb for humans, and for immature animals and dairy animals.

Assay Principles

The AgraQuant[®] Total Aflatoxin Assay is a direct competitive enzyme-linked immunosorbent assay (ELISA). Aflatoxins are extracted from a ground sample with 70% methanol. The extracted sample and enzyme-conjugated aflatoxin are mixed and added to the antibody-coated microwell. Aflatoxins in samples and control standards are allowed to compete with enzyme-conjugated aflatoxin for the antibody binding sites. After a washing step, an enzyme substrate is added and blue color develops. The intensity of the color is inversely proportional to the concentration of aflatoxin in the sample or standard. A stop solution is then added which changes the color from blue to yellow. The microwells are measured optically using a microwell reader with an absorbance filter of 450nm (OD₄₅₀) and a differential filter of 630nm. The optical densities of the samples are



compared to the OD's of the standards and an interpretative result is determined.

Precautions

1. Store reagents at 2-8°C (35-46°F) when not in use, and do not use beyond the expiration date.
2. Adhere to incubation times stated in the procedure. Use of incubation times other than those specified may give inaccurate results.
3. Methanol is flammable. Caution must be taken in its use and storage.
4. The Stop Solution contains acid. Avoid contact with skin or eyes. If exposed, flush with water.
5. Consider all materials, containers and devices that are exposed to the sample or standards to be contaminated with toxin. Wear protective gloves and safety glasses when using the kit.
6. Dispose of all materials, containers and devices appropriately after use.
7. The conjugate solution is colored green in order to help customers to distinguish whether conjugate was already added to microwells or not. The greenness of conjugate solution may vary among production batches, nevertheless, this does not affect the conjugate quality.



For further information please contact:

Technical Services
Romer Labs Singapore Pte. Ltd.
3791 Jalan Bukit Merah #08-08
e-Centre@redhill,
Singapore, 159471

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Web: <http://www.romerlabs.com>
Email: salesasia@romerlabs.com

Warranty

The user assumes all risk in using Romer Labs, Inc.[®] products and services. Romer Labs, Inc.[®] will warrant that its products and services meet all quality control standards set by Romer Labs, Inc.[®], and Romer Labs, Inc.[®] will, at its option, repair or replace any product, components, or repeat services which prove to be defective in workmanship or material within product specific warranty periods or expiration dates and which our examination shall disclose to our satisfaction to be defective as such. This warranty is expressly in lieu of all other warranties, expressed or implied, as to description, quality, merchantability, fitness for any particular purpose, productiveness, or any other matter. Romer Labs, Inc.[®] shall be in no way responsible for the proper use of its products. Romer Labs, Inc.[®] hereby disclaims all other remedies, warranties, guarantees or liabilities, expressed or implied, arising by law or otherwise, and it shall have no liability for any lost profits or damage, direct, indirect or otherwise, to person or property, in connection with the use of any of its products or services. This warranty shall not be extended, altered or varied except by a written instrument signed by an authorized representative of Romer Labs, Inc.[®]



AgraQuant® Ochratoxin
Assay 2/40

Order No.:
COKAQ2000/COKAQ2048

Romer Labs Singapore Pte. Ltd.

Tel: (65) 6631 8018

Fax: (65) 6275 5584

Web: <http://www.romerlabs.com>

This Package Insert is available in following languages:

- Portuguese
- Spanish
- French
- Chinese
- Polish

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For further information please contact:

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Email: salesasia@romerlabs.com



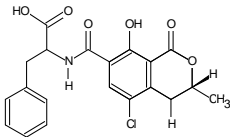
AgraQuant[®] Ochratoxin Assay 2/40 Competitive ELISA



Order #: COKAQ2000/COKAQ2048

Ochratoxin

Ochratoxin, produced mainly by the fungi *Aspergillus ochraceus* and *Penicillium verrucosum*, can be found in a wide variety of commodities such as raisins, barley, soy products and coffee, etc.



Ochratoxin A

Short Instruction:



1 Pipette 200 μ L conjugate solution into dilution wells

2 Add 100 μ L of each standard or sample extract into the dilution wells.

3 Mix well and transfer 100 μ L from dilution wells into antibody coated wells and incubate at RT for 10 minutes

4 Wash 5 times with distilled or deionized water

5 Tap dry washed wells

6 Pipette 100 μ L substrate solution into the antibody coated wells and incubate at RT for 5 minutes

7 Add 100 μ L stop solution into the antibody coated wells

8 Read the strips with ELISA reader using 450nm filter and 630nm differential filter



Performance Characteristics: LOD: 1.9 ppb LOQ: 2 ppb
Range: 2-40 ppb

Sample Preparation / Extraction

1. Obtain a representative sample and grind it using a Romer Series II® Mill so that 75% will pass through a 20-mesh screen, then thoroughly mix the subsample portion.
2. Weigh out 20 g of ground sample into a clean jar or a conic flask that can be tightly sealed.
3. Add 100 mL of 70/30 (v/v) methanol/water extraction solution and seal jar. Note: Samples should be extracted in a ratio of 1:5 (w:v) of sample to extraction solution respectively.
4. Shake or blend for 3 minutes.
5. Allow sample to settle, then filter the top layer of extract through a Whatman #1 filter and collect the filtrate.

Note: Commodity extracts should have a pH of 6-8. Excessive alkaline or acidic conditions may affect the test results and should be adjusted before testing.

For beer: pipette 3mL of a beer sample into a test tube; pipette 7mL of 100% methanol into the same tube; vortex or mix for 30 seconds. Sample is now ready for testing. The final result of ochratoxin in beer is calculated by multiplying a factor of (2/3) to the ELISA result.

For wine: pipette 3mL of a wine sample into a test tube; pipette 5.7mL of 100% methanol into the same tube; vortex and mix for 30 seconds; adjust pH value in the range of 6.5-7.5 using 1M NaOH; the sample is now ready for testing. The final result of ochratoxin in wine is calculated by multiplying a factor of 0.58 to the ELISA result.



Assay Procedure in Detail

Note: All reagents and kit components must be at room temperature 18-30°C (64-86°F) before use. It is recommended that an 8-channel pipettor be used to perform the assay. No more than 48 samples and standards total (6 test strips) should be run in one experiment when using an 8-channel pipettor. If an 8-channel pipettor is not used (i.e. using only single channel pipettes), it is recommended that no more than a total of 16 samples and standards (2 test strips) be run in any one experiment.

1. Place the appropriate number of blue/green-bordered Dilution Strips in a microwell strip holder. One Dilution Well will be required for each standard, (i.e. 0, 2, 5, 20 & 40 ppb) or sample.
2. Place an equal number of Antibody Coated Microwell strips in a microwell strip holder. Return unused microwell strips to the foil pouch with the desiccant packet and reseal pouch with tape.
3. Measure the required amount of Conjugate from the green-capped bottle (~240 μL /well or 2 mL/strip) and place in a separate container (e.g. reagent boat when using the 8-channel pipettor). Using an 8-channel pipette, dispense 200 μL of Conjugate into each blue/green-bordered Dilution Well.
4. Using a single channel pipettor, add 100 μL of each standard or sample into the appropriate Dilution Well containing 200 μL of Conjugate. Use a fresh pipette tip for each standard or sample. Note: Make sure the pipette tip has been completely emptied.
Using an 8-channel pipettor with fresh tips for each 8-well strip, mix each well by carefully pipetting it up and down 3 times and immediately transfer 100 μL of the contents



- from each Dilution Well into a corresponding Antibody Coated Microwell. Incubate at room temperature for 10 minutes. Note: Do not agitate the plate to mix as it may cause well-to-well contamination.
5. Empty the contents of the microwell strips into a waste container. Wash by filling each microwell with distilled or deionized water, and then dumping the water from the microwell strips. Repeat this step 4 times for a total of 5 washes. Note: Take care not to dislodge the strips from the holder during the wash procedure.
 6. Lay several layers of absorbent paper towels on a flat surface and tap microwell strips on towels to expel as much residual water as possible after the fifth wash. Dry the bottom of the microwells with a dry cloth or towel.
 7. Measure the required amount of Substrate from the blue-capped bottle (~120 μL /well or 1 mL/strip) and dispense into a separate container (e.g. reagent boat for an 8-channel pipettor). Pipette 100 μL of the Substrate into each microwell strip using an 8-channel pipettor. Incubate at room temperature for 5 minutes.
 8. Measure the required amount of Stop Solution from the red-capped bottle (~120 μL /well or 1 mL/strip) and dispense into a separate container (e.g. reagent boat for an 8-channel pipettor). Pipette 100 μL of Stop Solution into each microwell strip using an 8-channel pipettor. The color should change from blue to yellow.
 9. Read the strips with a microwell reader using a 450 nm filter with a differential filter of 630nm. Record OD readings for each microwell. Note: Air bubbles should be eliminated prior to reading strips as they may affect analytical results.



Additional Notes: Ratio of Conjugate to Standard/Sample should remain at 2:1, but volumes of Conjugate and Standards/Samples can be reduced, e.g. using 100 μ L and 50 μ L, respectively. The content to be transferred from dilution well to antibody coated well remains the same as 100 μ L. Do not return unused reagents to their original bottles. Carefully keep track of the position of Samples and Standards during the assay. Do not mix the assay microwells by shaking at any time during test.

Interpretation of Results

Using either the unmodified OD values or the OD values expressed as a percentage of the OD of the zero (0) standard, construct a dose-response curve using the five standards. Since the amount of ochratoxin in each standard is known, the unknowns can be measured by interpolation from this standard curve. Results can also be easily calculated using the Romer[®] Log/Logit spreadsheet that is provided (free of charge) upon request. If the Log/Logit regression model is used for results interpretation, the linearity coefficient (r^2) of the calibration curve should be no less than 0.985. An OD value of less than 0.5 absorbance units for 0ppb standard may indicate deterioration of reagents.

If a sample contains ochratoxin levels higher than the highest standard (>40 ppb), the filtered extract should be further diluted in 70% methanol such that the diluted sample results are in a range of 5 - 40 ppb and reanalyzed to obtain accurate results. The dilution factor must be included when the final result is calculated.



Performance Characteristics in Detail

Limit of detection (LOD): 1.9 ppb (Determined by the average values of 10 ochratoxin-free corn samples plus 2 standard deviation).

Limit of quantitation: 2 ppb (Described as the lowest concentration point on the calibration curve that this test can reliably detect ochratoxin).

Range of quantitation: 2 – 40 ppb (For quantitation of samples above 40 ppb samples should be diluted such that the diluted sample result are in a range of 5 - 40 ppb).



Materials supplied

Order #: COKAQ2000

- 96 antibody coated microwells (12 eight-well strips) in a microwell holder (sealed in a foil pouch)
- 96 non-coated dilution microwells (12 eight-well strips marked with blue/green at base)
- 5 vials of 1.5mL of each ochratoxin standard (0, 2, 5, 20 and 40 ppb)
- 1 bottle of 25mL of ochratoxin conjugate (green-capped bottle)
- 1 bottle of 15mL of substrate solution (blue-capped bottle)
- 1 bottle of 15mL of stop solution (red-capped bottle)

Order #: COKAQ2048

- 48 antibody coated microwells (6 eight-well strips) in a microwell holder (sealed in a foil pouch)
- 48 non-coated dilution microwells (6 eight-well strips marked with blue/green at base)
- 5 vials of 0.75mL of each ochratoxin standard (0, 2, 5, 20 and 40 ppb)
- 1 bottle of 12.5mL of ochratoxin conjugate (green-capped bottle)
- 1 bottle of 7.5mL of substrate solution (blue-capped bottle)
- 1 bottle of 7.5mL of stop solution (red-capped bottle)

Materials required but not supplied

Extraction Procedure

- *EQMMS2010: Romer Series II[®] Mill or equivalent
- *EQOLE1025: Blender or a tightly sealing jar with lid
- 250mL conic flask with plug
- *EQOLE1010: Balance, 400 g



- *EQOLE1050: Graduated cylinder: 100mL
- *100% methanol: ACS grade methano
- *70% methanol or
 - ACS grade methanol for making 70 % methanol
 - Distilled or de-ionized water for making 70 % methanol
- Container for filtrate collection (e.g. flask or falcon tubes)
- *Whatman#1 filter paper, or equivalent
- *Filter funnel

Assay Procedure

- *8-channel and single channel pipettors capable of pipetting 100 μ L and 200 μ L with tips
- *EQOLE1300: Timer
- *COKAD1150: Wash bottle
- Distilled or de-ionized water
- Absorbent paper towels
- *3 reagent boats for use as reagent containers for an 8-channel pipettor
- *Microwell reader with a 450nm filter and an optional differential filter of 630nm or equivalent.

*Items available from Romer Labs, Inc.[®] - Americas Division

Technical and Background Information

The AgraQuant[®] Ochratoxin Assay is a direct competitive enzyme-linked immunosorbent assay (ELISA) that determines a quantitative level for the presence of ochratoxin A and B and is intended for use in grains, cereals, nuts, animal feeds and other commodities.

The AgraQuant[®] Ochratoxin Assay has been validated for barley, beer, cocoa, corn, cereal, green coffee, milo, soybeans, wheat and wine.



Ochratoxin

Ochratoxin, produced mainly by the fungi *Aspergillus ochraceus* and *Penicillium verrucosum*, can be found in a wide variety of commodities such as raisins, barley, soy products and coffee, etc. Though the ochratoxin amounts may be relatively low, it is often not rapidly removed from the body and its levels may accumulate in the blood and other selected tissues of either humans or animals consuming contaminated food.

Ochratoxin is primarily a kidney toxin but if the concentration is sufficiently high, there can be damage to the liver as well. Ochratoxin is a carcinogen in rats and mice and is suspected to be the causative agent of a human disease, Balkan Endemic Nephropathy, which affects the kidneys. Often, tumors are associated with this disease.

Assay Principles

The AgraQuant[®] Ochratoxin Assay is a solid phase direct competitive enzyme immunoassay. Ochratoxin A/B is extracted from a ground sample with 70% methanol. The extracted sample and enzyme-conjugated ochratoxin are mixed and added to the antibody-coated microwell. Ochratoxin in samples and control standards are allowed to compete with enzyme-conjugated ochratoxin for the antibody binding sites. After a washing step, an enzyme substrate is added and blue color develops. The intensity of the color is inversely proportional to the concentration of ochratoxin in the sample or standard. A stop solution is then added which changes the color from blue to yellow. The microwells are measured optically by a microplate reader with an absorbance filter of 450nm and a differential filter of 630nm. The optical densities of the samples are compared to the OD's of the standards and an interpretative result is determined.

Precautions

1. Store reagents at 2-8°C (35-46°F) when not in use, and do not use beyond the expiration date.



2. Adhere to incubation times stated in the procedure. Use of incubation times other than those specified may give inaccurate results.
3. Methanol is flammable. Caution must be taken in its use and storage.
4. The Stop Solution contains acid. Avoid contact with skin or eyes. If exposed, flush with water.
5. Consider all materials, containers and devices that are exposed to the sample or standards to be contaminated with toxin. Wear protective gloves and safety glasses when using the kit.
6. Dispose of all materials, containers and devices appropriately after use.
7. The conjugate solution is colored green in order to help customers to distinguish whether conjugate was already added to microwells or not. The greenness of conjugate solution may vary among production batches, nevertheless, this does not affect the conjugate quality.

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Warranty

The user assumes all risk in using Romer Labs, Inc.[®] products and services. Romer Labs, Inc.[®] will warrant that its products and services meet all quality control standards set by Romer Labs, Inc.[®], and Romer Labs, Inc.[®] will, at its option, repair or replace any



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AgraQuant[®] T-2/HT-2 Toxin Assay 25/500

Order No.: COKAQ6100

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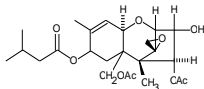
AgraQuant[®] T-2/HT-2 Toxin Assay 25/500 Competitive ELISA



Order #: COKAQ6100









T-2/HT-2 Toxin

T-2 and HT-2 toxins are type-A trichothecene mycotoxins, which are closely-related epoxy sesquiterpenoids. T-2 and HT-2 toxins are produced by fungi of the *Fusarium* genus, and the most important producer is *Fusarium sporotrichioides*. These mycotoxins occur in grains such as wheat, maize, oats, barley, rice, beans and soybeans as well as in some cereal-based products.



T-2 Toxin

Short Instruction:

-  Pipette **50 µL enzyme conjugate** into the microwells
-  Add **50 µL of each diluted standard or sample** into the microwells
-  Pipette **50 µL of antibody solution** into the microwells. Gently mix for 30 sec. and **incubate at RT for 10 mins**
-  **Wash 5 times** with distilled/deionized water
-  **Tap dry** washed wells
-  Pipette **100 µL substrate solution** into each microwells and incubate **at RT for 5 mins**
-  Add **100 µL stop solution** into the antibody coated wells
-  **Read** results at 450 nm with an ELISA reader

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PI_COKAQ6100_TSW_EN_v06

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Performance Characteristics:

LOD: 29 ppb (corn)

57 ppb (oats)

Range: 25 - 500 ppb (based on calibrators' concentration)

37 - 500ppb (corn)

72 - 500ppb (oats)

Sample Preparation / Extraction

1. Obtain a representative sample and grind it using a Romer Series II[®] Mill so that 95 % will pass through a 20-mesh screen, then thoroughly mix the subsample portion.
2. Weigh out 20 g of ground sample into a clean jar that can be tightly sealed.
3. Add 100 mL of 70 % methanol and seal jar. **Note:** Samples should be extracted in a ratio of 1:5 (w:v) of sample to extraction solution respectively.
4. Vigorously shake the jar for 3 minutes.
5. Allow sample to settle, then filter the top layer of extract through a Whatman #1 filter and collect the filtrate. **Note:** Commodity extracts should have a pH of 6-8. Excessive alkaline or acidic conditions may affect the test results and should be adjusted before testing.
6. Dilute the sample extract 1:10 with deionized or distilled water. For example, add 1 mL of extract to 9 mL of distilled or deionized water.
7. The sample is ready for testing without further preparation.



Assay Procedure in Detail

Note: All reagents and kit components must be at room temperature 18-30°C (64-86°F) before use. It is recommended that an 8-channel pipettor be used to perform the assay. No more than 48 samples and standards total (6 test strips) should be run in one experiment when using an 8-channel pipettor. If an 8-channel pipettor is not used (i.e. using only single channel pipettes), it is recommended that no more than a total of 16 samples and standards (2 test strips) be run in any one experiment.

1. Dilute kit standards (i.e. 0, 25, 100 & 500 ppb) 1:10 with deionized or distilled water in test tubes. For example, add 0.1 mL of standard to 0.9 mL of deionized or distilled water and mix.
2. Place the appropriate number of microwells into a microwell holder. Make sure to re-seal unused wells in the zip-lock bag with desiccant.
3. Pipette **50 µL of Enzyme conjugate** into each microwell.
4. Using a single channel pipettor, add **50 µL of each diluted standard or sample** to the appropriate microwell containing 50 µL of Enzyme conjugate. Use a fresh pipette tip for each standard or sample. **Note:** Make sure the pipette tip has been completely emptied.
5. Pipette **50 µL of Antibody Solution** into each microwell. Incubate at room temperature for **10 minutes** (Gently move the plate in a circular motion for 30 seconds to mix the contents).
6. Empty the contents of the microwell strips into a waste container. Wash by filling each microwell with distilled or deionized water, and then dumping the water from the microwell strips. Repeat this step 4 times for a total of 5 washes. **Note:** Take care not to dislodge the strips from the holder during the wash procedure.



7. Lay several layers of absorbent paper towels on a flat surface and tap microwell strips on towels to expel as much residual water as possible after the fifth wash. Dry the bottom of the microwells with a dry cloth or towel.
8. Measure the required amount of Substrate from the Substrate bottle ($\sim 120 \mu\text{L}/\text{well}$ or $1\text{mL}/\text{strip}$) and dispense into a separate container (e.g. reagent boat for an 8-channel pipettor). Pipette **100 μL of the Substrate** into each microwell strip using an 8-channel pipettor. Incubate at room temperature for **5 minutes**.
9. Measure the required amount of Stop Solution from the Stop Solution bottle ($\sim 120 \mu\text{L}/\text{well}$ or $1 \text{ mL}/\text{strip}$) and dispense into a separate container (e.g. reagent boat for an 8-channel pipettor). Pipette **100 μL of Stop Solution** into each microwell strip using an 8-channel pipettor. The color should change from blue to yellow.
10. Read the strips with a microwell reader using a 450 nm filter. Record OD readings for each microwell. **Note:** Air bubbles should be eliminated prior to reading strips as they may affect analytical results.



Interpretation of the Results

Using either the unmodified OD values or the OD values expressed as a percentage of the OD of the zero (0) standard, construct a dose-response curve using the four standards. Since the amount of T-2/HT-2 in each standard is known, the unknowns can be measured by interpolation from this standard curve. Results can also be easily calculated using the Romer[®] Log/Logit spreadsheet that is provided (free of charge) upon request. If the Log/Logit regression model is used for results interpretation, the linearity coefficient (r^2) of the calibration curve should be no less than 0.985.

An OD value of less than 0.5 absorbance units for 0 ppb standard may indicate deterioration of reagents.

Samples containing less than lower limit of quantitation (LLOQ) should be reported as "< LLOQ". Samples containing greater than 500 ppb should be reported as "> 500 ppb". Samples containing T2/HT2 greater than 500 ppb should be further diluted using 70% Methanol on the sample extracts after step 5 of the section "Sample Preparation/Extraction", and then according to step 6 further diluted 1:10 with deionized or distilled water such that the diluted sample results are within the range of quantitation and reanalyzed to obtain accurate results. The dilution factor must be included when the final result is calculated.

Performance Characteristics in Detail

Limit of detection: 29 ppb (corn)
57 ppb (oats)



Range of quantitation: 25 - 500 ppb (based on calibrators' concentration)
37 - 500ppb (corn)
72 - 500ppb (oats)

Cross Reactivity:

Compound	Cross reactivity %
T-2	100
HT-2	94
T-2 Triol	<2.0
T-2 Tetraol	<0.04
Verrucarol	<0.04

Materials supplied

- 96 antibody coated microwells (12 eight-well strips) in a microwell holder (sealed in a ziplock foil pouch).
- 4 vials of 2 mL of each T-2 toxin standard. Standard concentrations are 0, 25, 100 and 500ppb, respectively. Standards need further dilution of 1:10 with deionized or distilled water before assay.
- 1 bottle of 8 mL of Enzyme conjugated T2/HT-2 toxin
- 1 bottle of 8 mL of Anti-T-2/HT-2 antibody
- 1 bottle of 14 mL of substrate solution
- 1 bottle of 14 mL of stop solution

Materials required but not supplied

Extraction Procedure

- *EQMMS2010: Romer Series II® Mill or equivalent
- *EQOLE1025: Blender or a tightly sealing jar with lid
- *EQOLE1010: Balance, 400 g
- *EQOLE1050: Graduated cylinder: 100 mL



- Container with a minimum 125 mL capacity
- *Whatman #1 filter paper, or equivalent
- *Filter funnel

Assay Procedure

- *8-channel and single channel pipettors capable of pipetting 100 μ L and 200 μ L with tips
- *EQOLE1300: Timer
- *COKAD1150: Wash bottle
- Distilled or de-ionized water
- Absorbent paper towels
- *4 reagent boats for use as reagent containers for an 8-channel pipettor
- *Microwell reader with a 450 nm filter

*Items available from Romer Labs, Inc.[®] - Americas Division

Technical and Background Information

The AgraQuant[®] T-2/HT-2 Toxin Assay is a direct competitive enzymelinked immunosorbent assay (ELISA) that determines a quantitative level for the presence of T-2 and HT-2 toxin and is intended for use in corn, corn meal, corn germ meal, corn gluten meal and corn/soy blend.

T-2/HT-2 Toxin

T-2 and HT-2 toxins are type-A trichothecene mycotoxins, which are closely-related epoxy sesquiterpenoids. T-2 and HT-2 toxins are produced by fungi of the *Fusarium* genus, and the most important producer is *Fusarium sporotrichioides*. These mycotoxins occur in grains such as wheat, maize, oats, barley, rice, beans and soybeans as well as in some cereal-based products. T-2 and HT-2 toxins are not normally found in grain at harvest but result from water damage



to the grain such as may occur when it remains for extended periods in the field at or after harvest, especially in cold weather, or in grain that becomes wet during storage. T-2 toxin inhibits protein synthesis and affects the actively dividing cells such as those lining the gastrointestinal tract, skin, lymphoid and erythroid cells. The effects of T-2 toxin to animals include weight loss or poor weight gain, bloody diarrhea, dermal necrosis or beak lesions, hemorrhage and decreased production (weight gain, eggs, milk, etc.).

Assay Principles

The AgraQuant[®] T-2/HT-2 Toxin Assay is a direct competitive enzyme-linked immunosorbent assay (ELISA). T-2/HT-2 toxins are extracted from a ground sample with 70 % methanol. The extract is further diluted at 1:10 using de-ionized or distilled water. Enzyme conjugated T-2/HT-2 toxin is pipetted into the microwells followed by calibrators or sample extracts. T2/HT-2 toxin antibody is then pipetted into the microwells to initiate the reaction. T-2/HT-2 toxins from the sample and enzyme conjugated T-2/HT-2 toxin compete for binding to T2/HT-2 toxin antibody which, in turn, binds to the microwells. After the 10 minute incubation, the contents of the microwells are removed and the microwells are washed to remove any unbound enzyme conjugated toxin. An enzyme substrate is added and blue color develops. The intensity of the color is inversely proportional to the concentration of T-2/HT-2 toxin in the sample or standard. A stop solution is then added which changes the color from blue to yellow. The microwells are measured optically using a microwell reader with an absorbance filter of 450 nm. The optical densities (OD) of the samples are compared to the OD's of the standards and an interpretative result is determined.



Precautions

1. Store reagents at 2-8°C (35-46°F) when not in use, and do not use beyond the expiration date.
2. Adhere to incubation times stated in the procedure. Use of incubation times other than those specified may give inaccurate results.
3. The Stop Solution contains acid. Avoid contact with skin or eyes. If exposed, flush with water.
4. Consider all materials, containers and devices that are exposed to the sample or standards to be contaminated with toxin. Wear protective gloves and safety glasses when using the kit.
5. Dispose of all materials, containers and devices appropriately after use.

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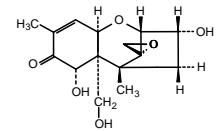
AgraStrip[®] Deoxynivalenol (DON) Quantitative Test



Order #: COKAS4000A

Intended Use

The AgraStrip[®] Deoxynivalenol (DON) Quantitative Test is a one-step lateral flow immunochromatographic assay that determines a quantitative level for the presence of deoxynivalenol and is intended for use in grains and grain products.



Deoxynivalenol

Deoxynivalenol

Deoxynivalenol (DON) is a type B trichothecene. DON is produced by fungi of the *Fusarium* genus, particularly *Fusarium graminearum*. This mycotoxin occurs predominantly in grains such as wheat, barley, oats, rye, and maize. DON is highly toxic, levels above 1 ppm are considered potentially harmful to swine. Pet foods prepared with wheat contaminated with DON have been involved in acute toxicities. DON is a known immunosuppressant and may cause kidney problems. Humans are thought to exhibit a similar vomiting syndrome when consuming DON-contaminated grain.

The US Food and Drug Association advisory levels for DON are as follows: (1) 1 ppm for finished wheat products for human consumption; (2) 5 ppm for grain and grain byproducts destined for swine and other animals; and not to exceed 1 ppm in the diets for swine and 2 ppm in the diets of other animals; (3) 10 ppm for grain and grain byproducts for ruminating beef and feedlot cattle older than 4 months and for chickens; and not to exceed 5 ppm in the diet.

The European Commission sets maximum levels of DON in foodstuffs in the EC regulation 1881/2006: (1) 1.25 ppm for unprocessed cereals other than durum wheat, oats and maize; (2) 1.75 ppm for unprocessed durum wheat and oats and unprocessed maize; (3) 0.75 ppm for cereals intended for direct human consumption, cereal flour, bran and germ as end product marketed for direct human consumption, and pasta (dry); (4) 0.5 ppm for bread (including small bakery wares), pastries, biscuits, cereal snacks and breakfast cereal; (5) 0.2 ppm for processed cereal-based foods and baby foods for infants and young children.

Assay Principles

The AgraStrip[®] DON Quantitative Test is a one-step lateral flow immunochromatographic assay for the quantitative screening of deoxynivalenol in samples. The test is based on a competition immunoassay format. Antibody-particle complex (conjugate) coated in a microwell is dissolved in assay diluents and mixed with sample extract. A DON strip is placed into the microwell. The mixed content is then wicked onto a membrane of the DON strip, which contains a test zone and a control zone. The test zone captures free antibody-particle complex (conjugate), allowing color particles to concentrate and form a visible line. The color intensity of the line is inversely proportional to the concentration of DON in the sample. The line is always visible in the control zone regardless of the presence of DON. The DON strips are measured using an AgraVision Reader and the results are determined.



Precautions

1. Store test kits at 2-8°C (35-46°F) when not in use, and do not use beyond the expiration date. Do not freeze. Do not leave it in direct sunlight.
2. Test strips must be kept inside their original tubes.
3. Conjugate coated microwells must be kept inside their original tubes.
4. All reagents must be at room temperature before assay is running.
5. Adhere to the instructions of test procedures.
6. Do not re-use test strips.
7. Consider all materials, containers and devices that are exposed to the sample to be contaminated with toxin. Wear protective gloves and safety glasses when using the kit.
8. The components in this test kit have been quality control tested as a standard batch unit. Do not mix components from different lot numbers.

Procedure

Sample Preparation / Extraction

Obtain a representative sample and grind it using a Romer Series II[®] Mill or equivalent so that 75% will pass through a 20-mesh screen, then thoroughly mix the subsample portion.

Wheat (Method 1):

1. Weigh out 10g of ground sample into a Whirl-Pak[®] bag.
2. Add 80mL of distilled or de-ionized water and close Whirl-Pak[®] bag. Note: Samples should be extracted in a ratio of 1:8 (w:v) of sample to extraction solution respectively.
3. Vigorously shake for 1 minute.
4. Allow sample to settle for 5 min to get supernatant.
5. The sample is ready for assay.

Corn (Method 2):

1. Weigh out 10g of ground sample into a Whirl-Pak[®] bag.
2. Add 40mL of distilled or de-ionized water and close Whirl-Pak[®] bag. Note: Samples should be extracted in a ratio of 1:4 (w:v) of sample to extraction solution respectively.
3. Vigorously shake for 1 minute.
4. Allow sample to settle for 5 min to get supernatant.
5. The sample is ready for assay.

Test Procedure

Note: All reagents and kit components must be at room temperature 20-24°C (68-75°F) before use. The temperature of AgraStrip[®] Incubator is set at 35°C. There are two assay buffer bottles provided (one is for testing method 1 samples and the other is for testing method 2 samples). Please use the correct assay buffer for testing.

1. Place Assay Buffer bottle in the AgraStrip[®] heat block in the AgraStrip[®] incubator and incubate at 35°C for 30 minutes. During shipment the Assay Buffer will precipitate and during this 30 minutes heat treatment it will completely re-dissolve. After the 30 minutes incubation shake the Assay Buffer bottle to properly mix its contents to be homogenous.

Note: It is recommended to switch on the incubator (including the Assay Buffer) in the morning and to keep it on throughout the whole day.



2. Place the cover of the heat block on the top of the heat block. Remove sealing tape of conjugate coated microwells, and place the appropriate number of conjugate coated microwells inside the heat block. Re-seal those un-used conjugate coated microwells.
3. Add 50 μL of Assay Buffer to each conjugate coated microwell. Place the cover back into the heat block to cover the microwells and incubate for 30 seconds.
4. Lift up the cover and immediately add 50 μL of sample extract into the Assay Buffer in each microwell, mix the content in each microwell by pipetting it up and down 10 times. Note: the coated conjugate in each microwell must be dissolved completely.
5. Put one test strip into one microwell. Place the cover back into the heat block to cover the microwells and test strips.
6. Allow the test strip to develop color for 3 minutes. Lift the heat block cover and place it on the top of the heat block.
7. Wipe the end of the strip test onto an absorbent paper and insert the strip into the strip holder/tray for reading.
8. Use the AgraVision Reader and immediately read the strip and interpret result. Note: Use the SD card supplied with the kit. Follow the instruction of AgraVision Reader to read the strips.

Note: after the test, the used microwells can be removed easily with a stick provided with the AgraStrip[®] heat block.

Interpretation of the Results

A color line always appears in the upper section of the test strip to indicate that the test strip is working properly. This line is the Control Line (C). A line in the lower section of the test strip indicates the test result. This line is the Test Line (T).

Invalid results: If there is no control line in the control zone, the test is invalid and the sample should be re-tested by using a valid test strip

Valid results: 2 lines are visible. The intensity of the line in the test zone is concentration dependent and must be measured with an AgraVision Reader.

Limit of Detection (LOD): 210 ppb (Wheat, Method 1)
 190 ppb (Corn, Method 2)

Limit of Quantitation (LOQ): 250 ppb (wheat, Method 1)
 240 ppb (corn, Method 2)

Quantitation Range: 0 – 5000 ppb

Note: AgraStrip[®] DON Quantitative Test with AgraVision Reader gives quantitative results in the above defined quantitation range. If the result is lower than the limit of quantitation (LOQ), it should be reported as "<LOQ". If the result is higher than the high limit of quantitation range (HLQ), the result should be reported as ">HLQ".

Materials Supplied With Kit

- 1 tube containing 24 DON test strips
- 1 tube containing 24 microwells coated with antibody particle complex (conjugate)
- 1 bottle of 1.7ml of Assay Buffer 1 (for testing Method 1 samples)
- 1 bottle of 1.7ml of Assay Buffer 2 (for testing Method 2 samples)
- 1 bag of 48 pipette tips
- 24 Whirl-Pak[®] bags
- 1 SD card for the AgraVision[™] Reader



Materials Required But Not Provided With Kit

Extraction Procedure

- *EQMMS2010: Romer Series II® Mill or equivalent
- *EQOLE1010: Balance, 400 g
- *EQOLE1050: Graduated cylinder: 100mL

Assay Procedure

- **Single channel pipette capable of pipetting up to 100µL with tips
- **EQOLE1300: Timer
- **EQASR1003: AgraVision Reader without printer or EQASR1000: AgraVision Reader with printer
- * EQASR1500: AgraStrip® Incubator
- **EQASR1005: AgraStrip® heat block with cover and a stick

*Items available from Romer Labs, Inc.®

**Items available from Romer Labs Singapore Pte Ltd

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Appendix V – Laboratory Protocols Generated/Modified for this Project

- V.1 General Laboratory Safety Precautions Concerning Mycotoxin Analysis
- V.2 Sampling Procedure
- V.3 Quick Start Guide for AgraVision Strip Reader
- V.4 Quick Start Guide for StatFax 4700 Microstrip Reader
- V.5 Sample Preparation and Test Procedures for Aflatoxin (Nuts and Raisins)
- V.6 Sample Preparation and Test Procedures for Aflatoxin (Wheat)
- V.7 Sample Preparation and Test Procedures for Deoxynivalenol (Wheat)
- V.8 Sample Preparation and Test Procedures for Ochratoxin A (Nuts and Raisins)
- V.9 Sample Preparation and Test Procedures for Ochratoxin A (Wheat)
- V.10 Sample Preparation and Test Procedures for T-2 (Wheat)
- V.11 Decontaminating and Disposing of Materials Used During Mycotoxin Analyses
- V.12 Disposal of Samples
- V.13 SOP for Data Collection, Handling and Storage



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UNIVERSITY OF
Nebraska
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PHL Innovation Lab
Afghanistan

TITLE: General Laboratory Safety Precautions Concerning Mycotoxin Analysis

Written by: Luis Sabillón
Effective date: 05/11/2015

Edited by: Andréia Bianchini
Version: 1

PURPOSE:

1. Ensure personnel safety while performing mycotoxin analysis.
2. To ensure that all hazardous waste related to mycotoxin analysis are properly and safely managed, from its generation through handling, storage and disposal.

SCOPE:

The procedures detailed herein apply to all personnel and visiting research staff working in the analysis of mycotoxins.

GENERIC SAFETY RISKS:

Working with solvents is one of the main hazards that you will face during mycotoxin analysis. Many organic solvents are highly flammable and can form explosive air-vapor mixtures; most organic solvents are harmful or toxic. Failure to handle solvents correctly may result in:

- Skin and eye irritation;
- Skin defatting or dermatitis from prolonged or repeated skin exposure;
- Central nervous system depression;
- Reproductive and fetal effects;
- Chronic toxic effects, such as liver or kidney effects, from skin contact or inhalation of solvent vapors;
- Acutely toxic effects, including blindness and death;
- Flash fires and explosions.

Moreover, all wheat/nuts/raisins samples suspected of being contaminated with mycotoxins must be handled with care, since mycotoxins are potent carcinogenic substances.

RESPONSIBILITIES:

Laboratory supervisors are responsible for ensuring that:

- Laboratory workers have been educated in relevant safety issues regarding the handling of organic solvents, mycotoxin standards and samples.
- Only appropriately trained individuals are allowed to work with organic solvents and mycotoxins.
- Adequately ventilated areas are available for extraction procedures utilizing organic solvents.
- Laboratory workers have the necessary personal protective equipment.
- Laboratory workers are familiar with the protocol in case of emergency.

Laboratory workers are responsible for ensuring that:

- They are satisfied that they have received adequate supervisory guidance/training for a procedure involving use of solvents and mycotoxins.
- They do not undertake a procedure involving solvents and mycotoxins without prior consideration of the hazards involved.
- They are aware of appropriate emergency procedures, the location of spill kits and their use before working with solvents and mycotoxins.
- They use due diligence and specified personal protective equipment/facilities when working with solvents and mycotoxins.

MATERIALS AND EQUIPMENT

- **EYE PROTECTION.** Eye protection is necessary for most solvent operations and should be mandatory in the research laboratory. Laboratory workers must wear safety glasses when working with solvents and mycotoxins.
- **RESPIRATOR/PROTECTIVE MASK.** Depending on the planned use of solvents and assessed risks, a chemical cartridge respirator may be required. When grinding the samples a respirator/protective mask must be worn to avoid inhalation of dust potentially contaminated with mycotoxins.
- **GLOVES.** Gloves shall be worn whenever organic solvents and mycotoxins are handled. Lightweight PVC gloves are sufficient to prevent incidental contact. Heavier nitrile gloves are required for cleaning up spills and are required whenever hands or fingers must be immersed in solvent. Gloves should be removed and left to ventilate in a fumehood when solvent is spilled on the gloves.
- **PROTECTIVE CLOTHING.** A lab coat is required for active bench work with solvents and mycotoxins, and when disposing of solvents in the waste containers. Standard polyester lab coats are suitable for protection against small laboratory splashes.
- **FUMEHOODS.** A fume hood should be used when dispensing solvents from the containers; do not dispense flammable solvent in the presence of apparatus that is hot or that may generate a spark.
- **SPILL MATERIALS.** Spill kits should be available to deal with laboratory spillages. Paper towels or adsorbent materials such as spill control pillows, and chemical resistant gloves should also be available.
- **HYPOCHLORITE BLEACH SOLUTIONS:** Bleach solutions should be available to decontaminate the workspace, mycotoxin spills and every material used after working with mycotoxins.
- **WASTE SOLVENT CONTAINERS.** Appropriate containers to collect liquid waste from mycotoxin extraction should be available. The liquid (i.e. organic solvent and water) must be compatible with container material (e.g. acids must not be placed in a metal container).



PHL Innovation Lab Afghanistan	
TITLE: Sampling Procedure Protocol	
Written by: <u>Luis Sabillon</u> Effective date: <u>05/11/2015</u>	Edited by: <u>Andréia Bianchini</u> Version: <u>1</u>

PURPOSE:

To describe how a sample has to be taken, to be representative of a specific lot.

BACKGROUND:

The first important task in the sampling process is related to the ability of the samplers to evaluate each case scenario that they may encounter in the field and make the best decision regarding how they can best take a small portion of that product and be able to represent the total. Therefore, the first step in this process is defining the **lot size**. A lot is whatever amount of product the sampler is trying to represent in a specific situation. Examples of “lots” would be:

Wheat and flour products

- a. The total amount of flour obtained after grinding what a farmer brought in to the Asiab mill;
- b. The total amount of flour produced in a day or half-day in a commercial mill;
- c. The total amount of flour or wheat a farmer have stored in their house (it could be a single bag or several bags);
- d. The total number of bags a vendor may have available at the market coming from a single source (i.e. received all at once from a specific region of Afghanistan or imported from a specific country) or the total number of bags a vendor may have of a specific type of flour/wheat.
- e. The total number of flour/wheat bags in a warehouse from a single source (i.e. received all at once from a specific region of Afghanistan or imported from a specific country);
- f. The total number of flour/wheat bags a warehouse may be storing, if there is no information that help further segregate those bags. An example of further segregation would be flour/wheat received from a single source or a specific harvesting year;

Raisins and Nuts

- a. The total amount of raisins or nuts drying at a small processor;
- b. The total amount of raisins or nuts processed in a day or half-day in a commercial facility;
- c. The total amount of raisins or nuts a farmer have stored in their house (it could be a single bag or several bags);
- g. The total number of bags a vendor may have available at the market coming from a single source (i.e. received all at once from a specific region of Afghanistan or imported from a specific country) or the total number of bags a vendor may have of a specific type of raisins or nuts (i.e. pistachio shelled or unshelled, walnuts, paper shell almonds or other variety).
- h. The total number of bags of raisins or nuts in a warehouse from a single source (i.e. received all at once from a specific region of Afghanistan or imported from a specific country) or type (i.e. dark raisins or yellow raisins, paper shell almonds or other types);
- i. The total number of bags of raisins or nuts a warehouse may be storing, if there is no information that help further segregate those bags. An example of further segregation would be product received from a single source, of a single variety or a specific harvesting year;

PROCEDURES:

1. For bagged or piled products (i.e. flour, grain, nuts, raisins)

In order to obtain a representative sample from a lot a sufficient number of sub-samples must be taken using the following rules:

- If there is only one bag/pile of product, randomly select at least 5 sampling points and take a sub-sample from each point.
- If there are up to 10 bags of product, take one sub-sample per bag.
- If there are between 11 to 100 bags of product, randomly select 10 bags and take one sub-sample per bag.

Note: In the case of Asiab mills, sub-samples should be taken at intervals during grinding (i.e. beginning of grinding of a wheat lot, middle of grinding and at the end of the process).

The sampling points must be evenly distributed over the total lot/pile surface according to a grid sampling pattern (figure 1). If samples are in bags, using a sampler, take sub-samples at regularly spaced intervals over a given space (lot). Choose an initial location at random, and then define the remaining sampling locations so that all locations are at regular intervals over an area; for example, at the points identified by the intersection of each line in the grid shown in figure 1. If samples are piled, and the use of the probe is not possible due to a low height of the material, then follow the same grid pattern but use a measuring cup to obtain the sub-samples.

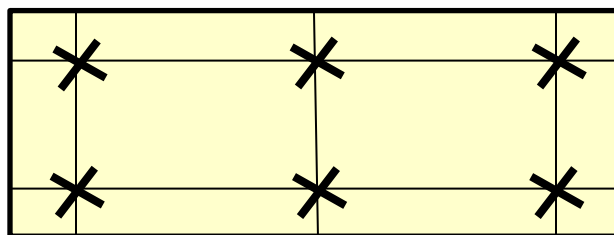


Figure 1. Grid Pattern

Note: If using the probe sampler, follow the steps listed from a through d (see figure 2):

- Insert the sampler into the product bag/container (A)
- Rotate the inner tube through 180° (B), to open the sampler. The product can now flow into the slot sampler.
- Rotate the inner tube through 180° to close the sampler and withdraw the sampler (C).
- Pull out the inner tube and deposit the sample into a plastic container (D).

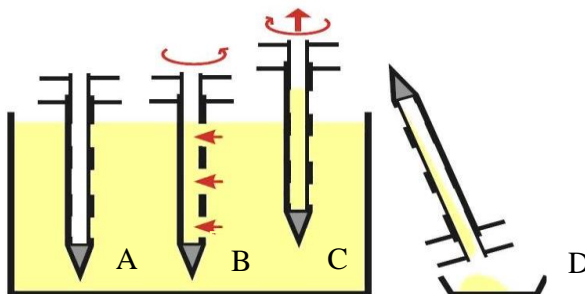


Figure 2. Using a sampler

2. For product spread out or hanging for drying (i.e. nuts and raisins)

In order to obtain a representative sample from a lot a sufficient number of sub-samples must be taken using the following rule:

- d. From the area where the product is drying randomly select at least 5 sampling points and take a sub-sample from each point. Figure 1 could be used as illustration of points for sampling almonds that may be spread out on the floor for drying or raisins that may be hanging from a wall.

3. Once all the sub-samples have been collected from the selected sampling points, thoroughly mix the sub-samples into a plastic container (tub or bucket) to obtain a composite sample.

4. From the composite sample, take the following number of sub-samples using a 1-liter plastic cup to obtain at least 500 g of shelled product:

- a) **Wheat flour:** 1 full cup
- b) **Almonds without shell:** 1 full cup
- c) **Almonds with shell:** 3 full cups
- d) **Walnuts without shell:** 2 full cups
- e) **Raisins:** 1 full cup
- f) **Pistachios without shell:** 1 full cup
- g) **Pistachio with shell:** 2 full cups

5. Place the 500 g sample in sterile plastics bags, properly labeled as shown in figure 3.

The image shows a white plastic bag with a yellow top, used for sample collection. A printed form is attached to the bag, titled "Feed the Future Innovation Lab for the Reduction of Post-Harvest Loss". The form includes the following fields and handwritten entries:

- Logos: USAID, Khyber Pakhtunkhwa, Nebraska
- Title: Feed the Future Innovation Lab for the Reduction of Post-Harvest Loss
- Section: Individual sample information
- Sample description: Wheat Raisins
- Date of sample collection: 3/20/15
- These samples have been stored (date a primary or reserve kit was used): 15
- Type of storage: Dry
- Sample origin: Kabul
- Location (if available):
- Product type: Rough Other
- or "Other" please specify:

Figure 3. Proper sample labeling

Note: The plastic cups must be cleaned after the sample is placed in the sterile plastic bag using wet wipes to remove any dust/particles adhered to the walls, followed by drying using paper towels.

6. Transfer samples to the laboratory in Kabul as soon as possible. In the meantime, store the samples in a clean dry place, away from pests such as insects, rodents or birds, until they are ready for shipment and further analysis. If extended storage (i.e., more than 3 days) is needed before sample is transferred to laboratory then samples should be frozen.

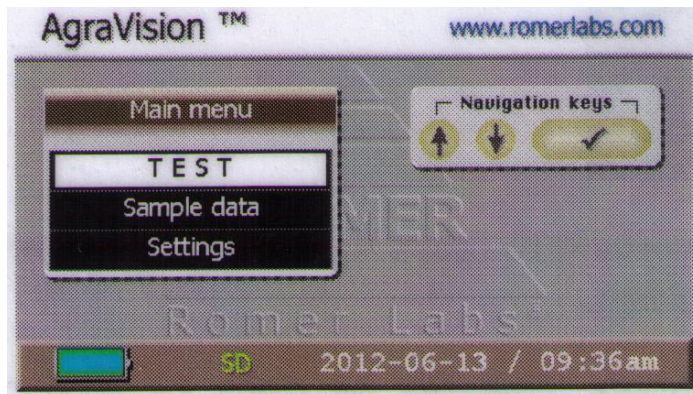
7. If samples are not shipped when expected, they must be stored in the freezer until the next day of shipping.

8. Sample information must be added to “*Sampling control log (AFG)*” prior to shipment.

PHL Innovation Lab Afghanistan	
Title: Quick Start Guide for AgraVision Strip Reader	
Commodity: Wheat	
Test: Deoxynivalenol	
Written by: <u>Luis Sabillón</u> Effective date: <u>05/11/2015</u>	Edited by: <u>Andréia Bianchini</u> Version: <u>1</u>

Procedure:

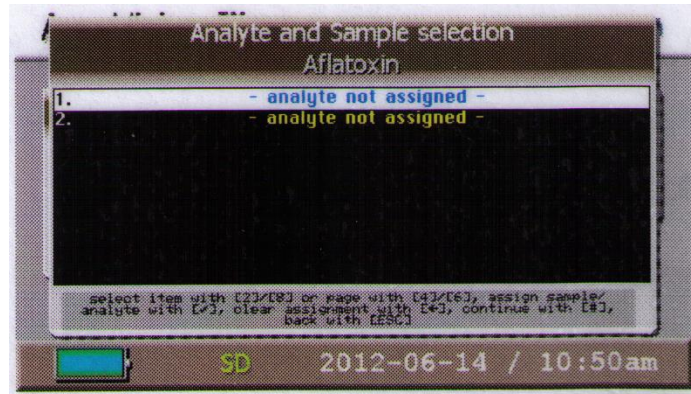
1. Switch ON the AgraVision Reader by pressing the Power button at the rear of the instrument.
2. Select “Test”



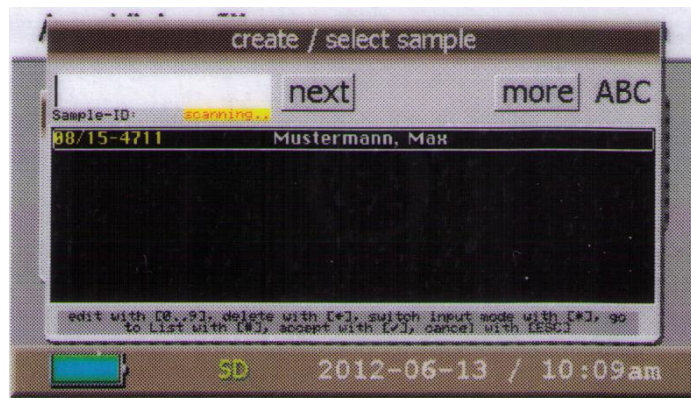
3. Select the designated analyte (e.g. Aflatoxin)



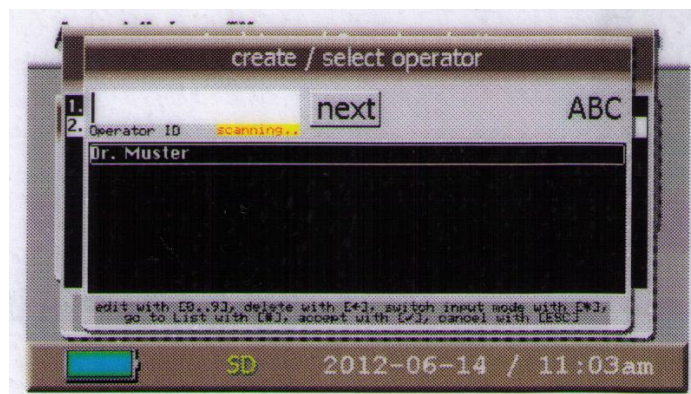
4. By pressing ↑ and ↓ move the flashing cursor to the strip position that corresponds to the position of the strip inserted into the tray.



5. Now scan the correct barcode (matrix dependent) from the AgraStrip tube. A beep sound will signal successful scanning.
6. A new window will open requesting a "Sample ID". Either key in a sample ID, scan a sample ID bar code or select from the menu listed on the screen. For selection of existing sample IDs press "# " and then press the "↓" key multiple times until you reach your designated sample ID.



7. Assign the next strip position according to steps 4, 5 and 6, or press the "# " key to skip the next tray position.
8. Now enter the operator information. This can be done by keying in an operator name/ID, scanning an operator ID bar code or selecting from the listed operators on the screen. For selection of existing operators press "# " and then press the "↓" key multiple times until you reach your designated operator.



9. Insert the mycotoxin tray with developed test strips and press the “✓” key to start the measurement.
10. Results will be displayed after less than 10 seconds. Print and/or save results.



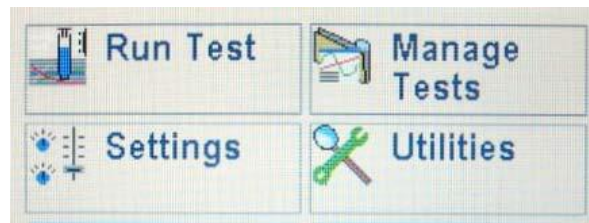
PHL Innovation Lab Afghanistan	
Title: Quick Start Guide for StatFax 4700 Microstrip Reader	
Commodity: Wheat/Nuts/Raisins	
Test: Aflatoxin/T2-HT2/Ochratoxin	
Written by: <u>Luis Sabillón</u> Effective date: <u>05/11/2015</u>	Edited by: <u>Andréia Bianchini</u> Version: <u>1</u>

Procedure:

1. Switch ON the StatFax 4700 – Microstrip Reader by pressing the Power button at the power supply module.



2. Select “Run Test” from the main display (touch screen).

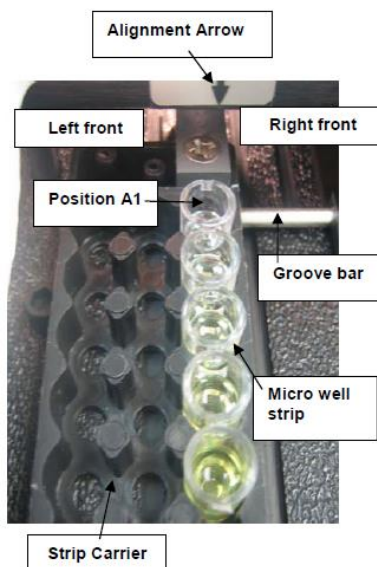


3. Tests programmed and saved on the instrument will display sorted by their test number. Each of the test parameters, including the mode, wavelengths, standards, units, and the ranges are all stored for reuse.

Note: If nothing has been programmed yet, the instrument will take you to the Create Test screen automatically (see Section 3.3.3 Manage Tests)

1 - T3	SAMPLE	Cancel	
2 - T4		^	<<
3 - TSH		V	>>
4 - TFSH/FSH VAST		Select	
5 - T3-Uptake		By #	
6 - LH/LH VAST			
7 - PRL			
8 - hCG			
9 - Digoxin			

4. Select and confirm a stored test (or create a new test) by using the following features:
 - **Arrows:** Use the ^ UP and V DOWN arrow keys to highlight selection; use the >> side arrows to advance to the next screen; use << arrows for previous screen.
 - **Select:** The highlighted test is executed once the Select key is pressed.
 - **Cancel:** Returns to the main power on display screen.
5. Once a test has been selected, the display will show the Auto-Track strip carrier, indicating where the standards and samples are located. At this time, select the number of wells or strips to be read.
6. Ensure that the wells are pushed down and seated firmly into the tray so that they will not cause the plate to jam on entry.
7. Use care that well tabs do not extend over other wells. Use caution when attaching labels so they do not jam in reader or interfere with read path. Before installing the strip carrier, note the location of the lead pin and groove on the carrier (Figure below); note the location of the alignment arrow inside the cover of the instrument (Figure below).



8. Place the strip carrier into the instrument with the lead pin first and the groove over the bar. Slide the carrier into position **all the way to left** side so that the instruments read position arrow align with position A1 on the strip (Figure above).
9. Once the first strip has been read, the software will build a calibration curve using the absorbance from the standards. Select “Print” to print out the standard curve along with absorbance from the samples.
10. Select “Continue” to proceed with the absorbance reading of the rest of the samples. Absorbance readings will be on the display for each well.
11. The message “Run another carrier of samples?” will appear. Choose whether or not to read more samples. If more samples are not going to be read, select “End” to finish the test.
12. Print/Save and interpret the results.

PHL Innovation Lab Afghanistan	
Title: Sample Preparation and Test Procedures for Aflatoxin	
Commodity: Raisins and Nuts (e.g., pistachios, almonds, walnuts)	
Test: AFLA Assay 4/40 (COKAQ1000/COKAQ1048)	
Written by: <u>Luis Sabillón</u> Effective date: <u>05/11/2015</u>	Edited by: <u>Andréia Bianchini</u> Version: <u>1</u>

Sample Preparation:

1. Obtain a representative sample of raisins or nuts using the *Sampling Procedure Protocol*.
2. If the sample still have their shells, they must be removed by hand. Only shelled samples should be used for analysis.

Note 1: From field samples, if shelled sample is around 500g, ensure that the whole sample is thoroughly mixed. The shelled sample is ready for analysis.

Note 2: From field samples, if shelled sample is around 1000g, now divide into two or more portions of at least 500 g each using the following procedure:

- a) Stack the sample evenly in a circular tray, spread the sample to cover the entire surface of the tray (**tray 1**).
- b) Place another circular tray (**tray 2**), facing down, of smaller diameter in the center (**see figure 1**). Discard the sample that have been left out of the tray of smaller diameter.

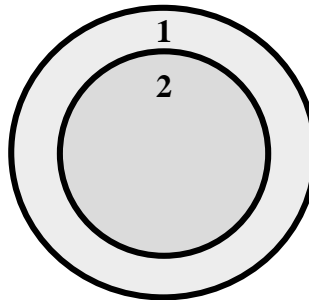


Figure 1. Procedure for reducing sample size.

3. Prepare the food processor by placing a clean workbowl on top of the motor axis and place the chopping blade inside of the workbowl.
4. From the sample left in **tray 2**, weigh out **300 g** of sample into the clean food processor workbowl.
5. Add enough water (distilled or de-ionized) to have a dilution ratio of 1:1.5 (sample: water) into the workbowl. In this case add **450ml** of water.

Note: The solid/liquid ratio should be kept at 1:1.5 (w:v).

6. Cover the workbowl with a plastic film before putting the lid. This will prevent the sample/water mixture from leaking.
7. Blend the mixture for 5 min at high speed.

Note: If needed, scrape any chunks that may have adhered to the wall, so that the mixture is ground evenly.

8. After slurry preparation, weigh out 20 g of mixed sample into a clean jar that can be tightly sealed, in duplicate.

Note: From this step forward the test needs to be done separate for each extract.

9. Add 100 mL of 70/30 (v/v) methanol/water extraction solution and seal jar.

Note: Samples should be extracted in a ratio of 1:5 (w:v) of sample to extraction solution respectively.

10. Blend for 3 minutes at medium speed.

11. Allow sample to settle, then filter the top layer of extract through a Whatman #1 filter and collect the filtrate.

12. Measure the pH of the extract. If needed, adjust pH value in the range of 6.0-8.0 using 1M NaOH or 1M Acetic acid.

Note: Commodity extracts should have a pH of 6.0-8.0. Excessive alkaline or acidic conditions may affect the test results and should be adjusted before testing.

13. **Except for walnuts**, the extracts need to be cleaned with a MycoSep 112 column before testing as follows (see figure 2):

13.1 Apply 4 ml extract to the glass tube.

13.2 Place the MycoSep column firmly into the top portion of the tube.

13.3 Push the MycoSep column into the tube, so the extract will pass through the column.

Note: You may tilt the tube slightly to wet the sides of the glass tube with the sample extract. This makes it easier to push the column into the tube.

13.4 The purified extract on the top is ready for testing.

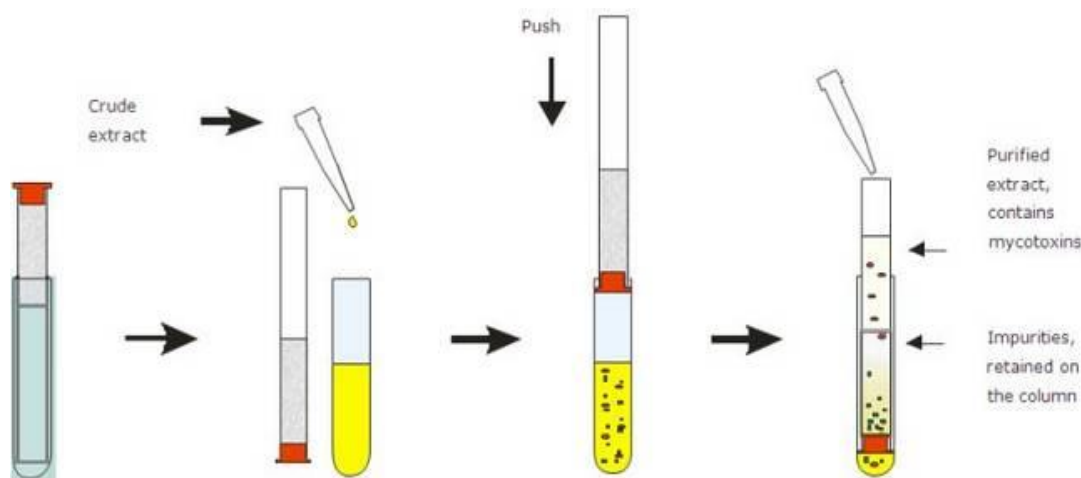


Figure 2. Procedure for using the MycoSep 112 columns

Test Procedure:

Note: All reagents and kit components must be at room temperature 18-30°C (64-86°F) before use. It is recommended that an 8-channel pipettor be used to perform the assay. No more than 48 samples and standards total (6 test strips) should be run in one experiment when using an 8-channel pipettor. If an 8-channel pipettor is not used (i.e. using only single channel pipettes), it is recommended that no more than a total of 16 samples and standards (2 test strips) be run in any one experiment.

1. Place the appropriate number of blue/green-bordered Dilution Strips in a microwell strip holder. One Dilution Well will be required for each standard, (i.e. 0, 4, 10, 20, & 40 ppb) or sample.

2. Place an equal number of Antibody Coated Microwell strips in a microwell strip holder. Return unused microwell strips to the foil pouch with the desiccant packet and reseal pouch with tape.
3. Measure the required amount of Conjugate from the greencapped bottle (~240 µL/well or 2 mL/strip) and place in a separate container (e.g. reagent boat when using the 8- channel pipettor). Using an 8-channel pipette, dispense 200 µL of Conjugate into each blue/green-bordered Dilution Well.
4. Using a single channel pipettor, add 100 µL of each standard or sample into the appropriate Dilution Well containing 200 µL of Conjugate. Use a fresh pipette tip for each standard or sample.

Note: Make sure the pipette tip has been completely emptied.

5. Using an 8-channel pipettor with fresh tips for each 8-well strip, mix each well by carefully pipetting it up and down 3 times and immediately transfer 100 µL of the contents from each Dilution Well into a corresponding Antibody Coated Microwell.
6. Incubate at room temperature for 15 minutes.

Note: Do not agitate the plate to mix as it may cause well-to-well contamination.

7. Empty the contents of the microwell strips into a waste container. Wash by filling each microwell with distilled or deionized water, and then dumping the water from the microwell strips. Repeat this step 4 times for a total of 5 washes.

Note: Take care not to dislodge the strips from the holder during the wash procedure.

8. Lay several layers of absorbent paper towels on a flat surface and tap microwell strips on towels to expel as much residual water as possible after the fifth wash. Dry the bottom of the microwells with a dry cloth or towel.
9. Measure the required amount of Substrate from the bluecapped bottle (~120 µL/well or 1 mL/strip) and dispense into a separate container (e.g. reagent boat for an 8- channel pipettor). Pipette 100 µL of the Substrate into each microwell strip using an 8-channel pipettor. Incubate at room temperature for 5 minutes. Measure the required amount of Stop Solution from the red-capped bottle (~120 µL/well or 1 mL/strip) and dispense into a separate container (e.g. reagent boat for an 8-channel pipettor). Pipette 100 µL of Stop Solution into each microwell strip using an 8-channel pipettor. The color should change from blue to yellow.
10. Read the strips with the **StatFax 4700** using a 450 nm filter with a 630nm differential filter. Record OD readings for each microwell. Refers to the Quick Start Guide for StatFax 4700 if needed.

Note: Air bubbles should be eliminated prior to reading strips as they may affect analytical results.

Additional Notes:

Ratio of Conjugate to Standard/Sample should remain at 2:1, but volumes of Conjugate and Standards/Samples can be reduced, e.g. using 100µL and 50µL, respectively. The content to be transferred from dilution well to antibody coated well remains the same as 100 µL. Do not return unused reagents to their original bottles. Carefully keep track of the position of Samples and Standards during the assay. Do not mix the assay microwells by shaking at any time during test.

Interpretation of Results:

Use the **Romer® Log/Logit AQ Afla_4-40 ppb spreadsheet** to interpret the results. Type the OD values obtained from each standard into the Section I of the spreadsheet to build the calibration curve. Make sure that the linearity coefficient (r²) of the calibration curve is no less than 0.985. Type the sample ID and the OD values obtained from each sample into the Section II of the spreadsheet to calculate the actual aflatoxin concentration. The final result of aflatoxin in sample is calculated by multiplying a dilution factor of 2.5 to the ELISA result.

Decision Making:

- If a sample contains aflatoxin levels higher than the highest standard (>40 ppb), the filtered extract should be further diluted in 70% methanol such that the diluted sample results are in a range of 5 – 20 ppb and reanalyzed to obtain accurate results. The dilution factor must be included when the final result is calculated.
- If a sample contains aflatoxin levels below the **limit of quantification (LOQ)** of 4 ppb, then assign a LOQ/2 (in this case would be 2 ppb) for the purpose of calculating the averages.
- If a sample contains aflatoxin levels below the **limit of detection (LOD)** of 3 ppb, then assign a value of zero (0) for the purpose of calculating the averages.
- If a sample contains aflatoxin levels above the quantitation range of the test of 40 ppb, a proper dilution must be made to ensure that the value falls within the range of 4 – 40 ppb.

Note: An OD value of less than 0.5 absorbance units for 0ppb standard may indicate deterioration of reagents.



PHL Innovation Lab Afghanistan	
Title: Sample Preparation and Test Procedures for Aflatoxin	
Commodity: Wheat	
Test: AFLA Assay 4/40 (COKAQ1000/COKAQ1048)	
Written by: <u>Luis Sabillón</u> Effective date: <u>05/11/2015</u>	Edited by: <u>Andréia Bianchini</u> Version: <u>1</u>

Sample Preparation:

1. Obtain a representative sample of wheat flour (or wheat grain) using the Sampling Procedure Protocol.

Note 1: From field samples, if wheat is already ground take a portion of the sample and evaluate the particle size by passing through a 20-mesh screen. If 95% of the sample passes through the sieve, the sample is ready for analysis. If not, the whole sample should be milled using a Romer Series II® Mill.

Note 2: From field samples, if wheat grain is obtained grind the whole sample using a Romer Series II® Mill so that 95 % will pass through a 20-mesh screen, then thoroughly mix the subsample portion.

2. When samples are ready for analysis, ensure that the whole sample is thoroughly mixed.
3. Weigh out 20 g of ground sample into a clean jar that can be tightly sealed, in duplicate.

Note: All remaining sample not used for analysis must be discarded according to the protocol Disposal of Samples.

Note: From this step forward the test needs to be done separate for each extract.

4. Add 100 mL of 70/30 (v/v) methanol/water extraction solution and seal jar.

Note: Samples should be extracted in a ratio of 1:5 (w:v) of sample to extraction solution respectively.

5. Blend for 3 minutes at medium speed.
6. Allow sample to settle, then filter the top layer of extract through a Whatman #1 filter and collect the filtrate.
7. Measure the pH of the extract. If needed, adjust pH value in the range of 6.0-8.0 using 1M NaOH or 1M Acetic acid.

Note: Commodity extracts should have a pH of 6.0-8.0. Excessive alkaline or acidic conditions may affect the test results and should be adjusted before testing.

8. Proceed to clean the sample extract using a MycoSep 112 column as follows (**see figure 1**):
 - 8.1 Apply 4 ml extract to the glass tube.
 - 8.2 Place the MycoSep column firmly into the top portion of the tube.
 - 8.3 Push the MycoSep column into the tube, so the extract will pass through the column.

Note: You may tilt the tube slightly to wet the sides of the glass tube with the sample extract. This makes it easier to push the column into the tube.

- 8.4 The purified extract on the top is ready for testing.

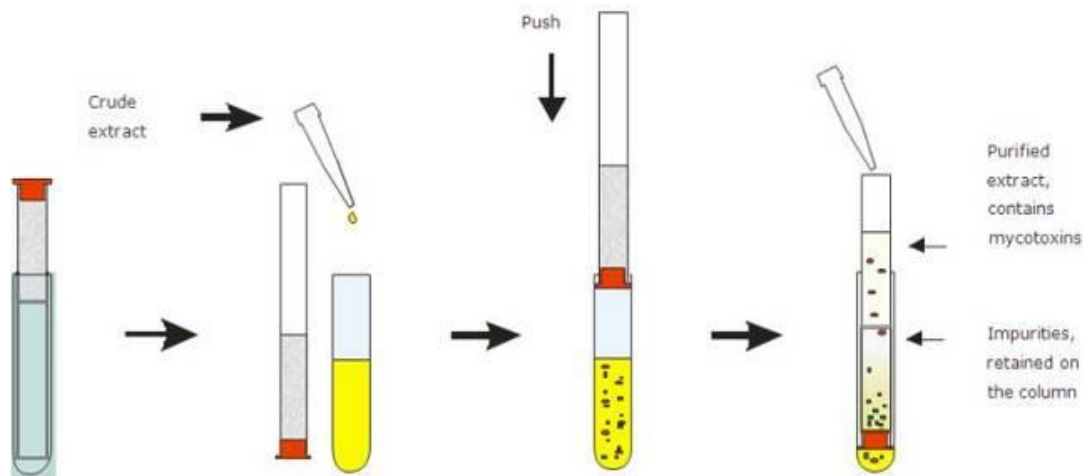


Figure 1. Procedure for using the MycoSep 112 columns

Test Procedure:

Note: All reagents and kit components must be at room temperature 18-30°C (64-86°F) before use. It is recommended that an 8-channel pipettor be used to perform the assay. No more than 48 samples and standards total (6 test strips) should be run in one experiment when using an 8-channel pipettor. If an 8-channel pipettor is not used (i.e. using only single channel pipettes), it is recommended that no more than a total of 16 samples and standards (2 test strips) be run in any one experiment.

1. Place the appropriate number of blue/green-bordered Dilution Strips in a microwell strip holder. One Dilution Well will be required for each standard, (i.e. 0, 4, 10, 20, & 40 ppb) or sample.
2. Place an equal number of Antibody Coated Microwell strips in a microwell strip holder. Return unused microwell strips to the foil pouch with the desiccant packet and reseal pouch with tape.
3. Measure the required amount of Conjugate from the greencapped bottle (~240 µL/well or 2 mL/strip) and place in a separate container (e.g. reagent boat when using the 8- channel pipettor). Using an 8-channel pipette, dispense 200 µL of Conjugate into each blue/green-bordered Dilution Well.
4. Using a single channel pipettor, add 100 µL of each standard or sample into the appropriate Dilution Well containing 200 µL of Conjugate. Use a fresh pipette tip for each standard or sample.

Note: Make sure the pipette tip has been completely emptied.

5. Using an 8-channel pipettor with fresh tips for each 8-well strip, mix each well by carefully pipetting it up and down 3 times and immediately transfer 100 µL of the contents from each Dilution Well into a corresponding Antibody Coated Microwell.
6. Incubate at room temperature for 15 minutes.

Note: Do not agitate the plate to mix as it may cause well-to-well contamination.

7. Empty the contents of the microwell strips into a waste container. Wash by filling each microwell with distilled or deionized water, and then dumping the water from the microwell strips. Repeat this step 4 times for a total of 5 washes.

Note: Take care not to dislodge the strips from the holder during the wash procedure.

8. Lay several layers of absorbent paper towels on a flat surface and tap microwell strips on towels to expel as much residual water as possible after the fifth wash. Dry the bottom of the microwells with a dry cloth or towel.

9. Measure the required amount of Substrate from the bluecapped bottle (~120 μL /well or 1 mL/strip) and dispense into a separate container (e.g. reagent boat for an 8- channel pipettor). Pipette 100 μL of the Substrate into each microwell strip using an 8-channel pipettor. Incubate at room temperature for 5 minutes.
10. Measure the required amount of Stop Solution from the red-capped bottle (~120 μL /well or 1 mL/strip) and dispense into a separate container (e.g. reagent boat for an 8-channel pipettor). Pipette 100 μL of Stop Solution into each microwell strip using an 8-channel pipettor. The color should change from blue to yellow.
11. Read the strips with the StatFax 4700 using a 450 nm filter with a 630nm differential filter. Record OD readings for each microwell.

Note: Air bubbles should be eliminated prior to reading strips as they may affect analytical results.

Additional Notes:

Ratio of Conjugate to Standard/Sample should remain at 2:1, but volumes of Conjugate and Standards/Samples can be reduced, e.g. using 100 μL and 50 μL , respectively. The content to be transferred from dilution well to antibody coated well remains the same as 100 μL . Do not return unused reagents to their original bottles. Carefully keep track of the position of Samples and Standards during the assay. Do not mix the assay microwells by shaking at any time during test.

Interpretation of Results:

Use the **Romer® Log/Logit AQ Afla_4-40 ppb spreadsheet** to interpret the results. Type the OD values obtained from each standard into the Section I of the spreadsheet to build the calibration curve. Make sure that the linearity coefficient (r^2) of the calibration curve is no less than 0.985. Type the sample ID and the OD values obtained from each sample into the Section II of the spreadsheet to calculate the actual aflatoxin concentration.

Decision Making:

- If a sample contains aflatoxin levels higher than the highest standard (>40 ppb), the filtered extract should be further diluted in 70% methanol such that the diluted sample results are in a range of 5 – 20 ppb and reanalyzed to obtain accurate results. The dilution factor must be included when the final result is calculated.
- If a sample contains aflatoxin levels below the **limit of quantification (LOQ)** of 4 ppb, then assign a LOQ/2 (in this case would be 2 ppb) for the purpose of calculating the averages.
- If a sample contains aflatoxin levels below the **limit of detection (LOD)** of 3 ppb, then assign a value of zero (0) for the purpose of calculating the averages.

Note: An OD value of less than 0.5 absorbance units for 0ppb standard may indicate deterioration of reagents.



PHL Innovation Lab Afghanistan	
Title: Sample Preparation and Test Procedures for Deoxynivalenol	
Commodity: Wheat	
Test: DON (COKAS4000A)	
Written by: <u>Luis Sabillón</u> Effective date: <u>05/11/2015</u>	Edited by: <u>Andréia Bianchini</u> Version: <u>1</u>

Sample Preparation:

1. Obtain a representative sample of wheat flour (or wheat grain) using the Sampling Procedure Protocol.

Note 1: From field samples, if wheat is already ground take a portion of the sample and evaluate the particle size by passing through a 20-mesh screen. If 95% of the sample passes through the sieve, the sample is ready for analysis. If not, the whole sample should be milled using a Romer Series II® Mill.

Note 2: From field samples, if wheat grain is obtained grind the whole sample using a Romer Series II® Mill so that 95 % will pass through a 20-mesh screen, then thoroughly mix the subsample portion.

2. When samples are ready for analysis, ensure that the whole sample is thoroughly mixed.
3. Weigh out 10 g of ground sample into a clean jar that can be tightly sealed, in duplicate.

Note: All remaining sample not used for analysis must be discarded according to the protocol Disposal of Samples.

Note: From this step forward the test needs to be done separate for each extract.

4. Add 80 mL of distilled or de-ionized water and seal jar.

Note: Samples should be extracted in a ratio of 1:8 (w:v) of sample to extraction solution respectively.

5. Blend for 1 minutes at medium speed.
6. Allow sample to settle for 5 min to get supernatant.
7. The sample is now ready for testing.

Test Procedure:

Note: All reagents and kit components must be at room temperature 20-24°C (68-75°F) before use. The temperature of AgraStrip® Incubator is set at 35°C. There are two assay buffer bottles provided (one is for testing method 1 samples (wheat) and the other is for testing method 2 samples (corn)). Please use the correct assay buffer for testing.

1. Place Assay Buffer bottle in the AgraStrip® heat block in the AgraStrip® incubator and incubate at 35°C for 30 minutes. During shipment the Assay Buffer will precipitate and during this 30 minutes heat treatment it will completely re-dissolve. After the 30 minutes incubation shake the Assay Buffer bottle to properly mix its contents to be homogenous.

Note: It is recommended to switch on the incubator (including the Assay Buffer) in the morning and to keep it on throughout the whole day.

2. Place the cover of the heat block on the top of the heat block. Remove sealing tape of conjugate coated microwells, and place the appropriate number of conjugate coated microwells inside the heat block. Re-seal those un-used conjugate coated microwells.

3. Add 50 μL of Assay Buffer to each conjugate coated microwell. Place the cover back into the heat block to cover the microwells and incubate for 30 seconds.
4. Lift up the cover and immediately add 50 μL of sample extract into the Assay Buffer in each microwell, mix the content in each microwell by pipetting it up and down 10 times.

Note: The coated conjugate in each microwell must be dissolved completely.

5. Put one test strip into one microwell. Place the cover back into the heat block to cover the microwells and test strips.
6. Allow the test strip to develop color for 3 minutes. Lift the heat block cover and place it on the top of the heat block.
7. Wipe the end of the strip test onto an absorbent paper and insert the strip into the strip holder/tray for reading.
8. Use the AgraVision Reader and immediately read the strip and interpret result. **Note:** Use the SD card supplied with the kit. Follow the instruction of AgraVision Reader to read the strips.

Note: After the test, the used microwells can be removed easily with a stick provided with the AgraStrip® heat block.

Interpretation of the Results:

A color line always appears in the upper section of the test strip to indicate that the test strip is working properly. This line is the Control Line (C). A line in the lower section of the test strip indicates the test result. This line is the Test Line (T).

Invalid results: If there is no control line in the control zone, the test is invalid and the sample should be re-tested by using a valid test strip.

Valid results: 2 lines are visible. The intensity of the line in the test zone is concentration dependent and must be measured with an AgraVision Reader.

Decision Making:

- If a sample contains DON levels below the **limit of quantification (LOQ)** of 250 ppb, then assign a LOQ/2 (in this case would be 125 ppb) for the purpose of calculating the averages.
- If a sample contains DON levels below the **limit of detection (LOD)** of 210 ppb, then assign a value of zero (0) for the purpose of calculating the averages.
- If a sample contains DON levels above the quantitation range of the test of 5000 ppb, a proper dilution must be made to ensure that the value falls within the range of 0 – 5000 ppb.

PHL Innovation Lab Afghanistan	
Title: Sample Preparation and Test Procedures for Ochratoxin	
Commodity: Raisins and Nuts (e.g., pistachios, almonds, walnuts)	
Test: OTA Assay 2/40 (COKAQ2000/COKAQ2048)	
Written by: <u>Luis Sabillón</u> Effective date: <u>05/11/2015</u>	Edited by: <u>Andréia Bianchini</u> Version: <u>1</u>

Sample Preparation:

1. Obtain a representative sample of raisins or nuts using the *Sampling Procedure Protocol*.
2. If the sample still have their shells, they must be removed by hand. Only shelled samples should be used for analysis.

Note 1: From field samples, if shelled sample is around 500g, ensure that the whole sample is thoroughly mixed. The shelled sample is ready for analysis.

Note 2: From field samples, if shelled sample is around 1000g, now divide into two or more portions of at least 500 g each using the following procedure:

- a) Stack the sample evenly in a circular tray, spread the sample to cover the entire surface of the tray (**tray 1**).
- b) Place another circular tray (**tray 2**), facing down, of smaller diameter in the center (**see figure 1**). Discard the sample that have been left out of the tray of smaller diameter.

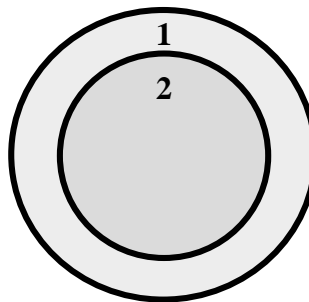


Figure 1. Procedure for reducing sample size.

3. Prepare the food processor by placing a clean workbowl on top of the motor axis and place the chopping blade inside of the workbowl.
4. From the sample left in **tray 2**, weigh out **300 g** of sample into the clean food processor workbowl.
5. Add enough water (distilled or de-ionized) to have a dilution ratio of 1:1.5 (sample: water) into the workbowl. In this case add **450ml** of water.

Note: The solid/liquid ratio should be kept at 1:1.5 (w:v).

6. Cover the workbowl with a plastic film before putting the lid. This will prevent the sample/water mixture from leaking.
7. Blend the mixture for 5 min at high speed.

Note: If needed, scrape any chunks that may have adhered to the wall, so that the mixture is ground evenly.

8. After slurry preparation, weigh out 20 g of mixed sample into a clean jar that can be tightly sealed, in duplicate.

Note: From this step forward the test needs to be done separate for each extract.

9. Add 100 mL of 70/30 (v/v) methanol/water extraction solution and seal jar.

Note: Samples should be extracted in a ratio of 1:5 (w:v) of sample to extraction solution respectively.

10. Blend for 3 minutes at medium speed.
11. Allow sample to settle, then filter the top layer of extract through a Whatman #1 filter and collect the filtrate.
12. Measure the pH of the extract. If needed, adjust pH value in the range of 6.0-8.0 using 1M NaOH or 1M Acetic acid.

Note: Commodity extracts should have a pH of 6.0-8.0. Excessive alkaline or acidic conditions may affect the test results and should be adjusted before testing.

Test Procedure:

Note: All reagents and kit components must be at room temperature 18-30°C (64-86°F) before use. It is recommended that an 8-channel pipettor be used to perform the assay. No more than 48 samples and standards total (6 test strips) should be run in one experiment when using an 8-channel pipettor. If an 8-channel pipettor is not used (i.e. using only single channel pipettes), it is recommended that no more than a total of 16 samples and standards (2 test strips) be run in any one experiment.

1. Place the appropriate number of blue/green-bordered Dilution Strips in a microwell strip holder. One Dilution Well will be required for each standard, (i.e. 0, 2, 5, 20 & 40 ppb) or sample.
2. Place an equal number of Antibody Coated Microwell strips in a microwell strip holder. Return unused microwell strips to the foil pouch with the desiccant packet and reseal pouch with tape.
3. Measure the required amount of Conjugate from the greencapped bottle (~240 µL/well or 2 mL/strip) and place in a separate container (e.g. reagent boat when using the 8- channel pipettor). Using an 8-channel pipette, dispense 200 µL of Conjugate into each blue/green-bordered Dilution Well.
4. Using a single channel pipettor, add 100 µL of each standard or sample into the appropriate Dilution Well containing 200 µL of Conjugate. Use a fresh pipette tip for each standard or sample.

Note: Make sure the pipette tip has been completely emptied.

5. Using an 8-channel pipettor with fresh tips for each 8-well strip, mix each well by carefully pipetting it up and down 3 times and immediately transfer 100 µL of the contents from each Dilution Well into a corresponding Antibody Coated Microwell.
6. Incubate at room temperature for 10 minutes.

Note: Do not agitate the plate to mix as it may cause well-to-well contamination.

7. Empty the contents of the microwell strips into a waste container. Wash by filling each microwell with distilled or deionized water, and then dumping the water from the microwell strips. Repeat this step 4 times for a total of 5 washes.

Note: Take care not to dislodge the strips from the holder during the wash procedure.

8. Lay several layers of absorbent paper towels on a flat surface and tap microwell strips on towels to expel as much residual water as possible after the fifth wash. Dry the bottom of the microwells with a dry cloth or towel.
9. Measure the required amount of Substrate from the bluecapped bottle (~120 µL/well or 1 mL/strip) and dispense into a separate container (e.g. reagent boat for an 8- channel pipettor). Pipette 100 µL of the Substrate into each microwell strip using an 8-channel pipettor. Incubate at room temperature for 5 minutes.

10. Measure the required amount of Stop Solution from the red-capped bottle (~120 µL/well or 1 mL/strip) and dispense into a separate container (e.g. reagent boat for an 8-channel pipettor). Pipette 100 µL of Stop Solution into each microwell strip using an 8-channel pipettor. The color should change from blue to yellow.
11. Read the strips with the **StatFax 4700** using a 450 nm filter with a differential filter of 630nm. Record OD readings for each microwell. Refers to the Quick Start Guide for StatFax 4700 if needed.

Note: Air bubbles should be eliminated prior to reading strips as they may affect analytical results.

Additional Notes:

Ratio of Conjugate to Standard/Sample should remain at 2:1, but volumes of Conjugate and Standards/Samples can be reduced, e.g. using 100µL and 50µL, respectively. The content to be transferred from dilution well to antibody coated well remains the same as 100 µL. Do not return unused reagents to their original bottles. Carefully keep track of the position of Samples and Standards during the assay. Do not mix the assay microwells by shaking at any time during test.

Interpretation of Results:

Use the **Romer® Log/Logit AQ OTA_2-40 ppb spreadsheet** to interpret the results. Type the OD values obtained from each standard into the Section I of the spreadsheet to build the calibration curve. Make sure that the linearity coefficient (r^2) of the calibration curve is no less than 0.985. Type the sample ID and the OD values obtained from each sample into the Section II of the spreadsheet to calculate the actual ochratoxin concentration. The final result of ochratoxin in sample is calculated by multiplying a dilution factor of 2.5 to the ELISA result.

Decision Making:

- If a sample contains ochratoxin levels higher than the highest standard (>40 ppb), the filtered extract should be further diluted in 70% methanol such that the diluted sample results are in a range of 5 - 40 ppb and reanalyzed to obtain accurate results. The dilution factor must be included when the final result is calculated.
- If a sample contains ochratoxin levels below the **limit of quantification (LOQ)** of 2 ppb, then assign a LOQ/2 (in this case would be 1 ppb) for the purpose of calculating the averages.
- If a sample contains ochratoxin levels below the **limit of detection (LOD)** of 1.9 ppb, then assign a value of zero (0) for the purpose of calculating the averages.

Note: An OD value of less than 0.5 absorbance units for 0ppb standard may indicate deterioration of reagents.



PHL Innovation Lab Afghanistan	
Title: Sample Preparation and Test Procedures for Ochratoxin	
Commodity: Wheat	
Test: OTA Assay 2/40 (COKAQ2000/COKAQ2048)	
Written by: <u>Luis Sabillón</u> Effective date: <u>05/11/2015</u>	Edited by: <u>Andréia Bianchini</u> Version: <u>1</u>

Sample Preparation:

1. Obtain a representative sample of wheat flour (or wheat grain) using the Sampling Procedure Protocol.

Note 1: From field samples, if wheat is already ground take a portion of the sample and evaluate the particle size by passing through a 20-mesh screen. If 95% of the sample passes through the sieve, the sample is ready for analysis. If not, the whole sample should be milled using a Romer Series II® Mill.

Note 2: From field samples, if wheat grain is obtained grind the whole sample using a Romer Series II® Mill so that 95 % will pass through a 20-mesh screen, then thoroughly mix the subsample portion.

2. When samples are ready for analysis, ensure that the whole sample is thoroughly mixed.
3. Weigh out 20 g of ground sample into a clean jar that can be tightly sealed, in duplicate.

Note: All remaining sample not used for analysis must be discarded according to the protocol Disposal of Samples.

Note: From this step forward the test needs to be done separate for each extract.

4. Add 100 mL of 70/30 (v/v) methanol/water extraction solution and seal jar.

Note: Samples should be extracted in a ratio of 1:5 (w:v) of sample to extraction solution respectively.

5. Blend for 3 minutes at medium speed.
6. Allow sample to settle, then filter the top layer of extract through a Whatman #1 filter and collect the filtrate.
7. Measure the pH of the extract. If needed, adjust pH value in the range of 6.0-8.0 using 1M NaOH or 1M Acetic acid.

Note: Commodity extracts should have a pH of 6.0-8.0. Excessive alkaline or acidic conditions may affect the test results and should be adjusted before testing.

8. The sample is now ready for testing.

Test Procedure:

Note: All reagents and kit components must be at room temperature 18-30°C (64-86°F) before use. It is recommended that an 8-channel pipettor be used to perform the assay. No more than 48 samples and standards total (6 test strips) should be run in one experiment when using an 8-channel pipettor. If an 8-channel pipettor is not used (i.e. using only single channel pipettes), it is recommended that no more than a total of 16 samples and standards (2 test strips) be run in any one experiment.

1. Place the appropriate number of blue/green-bordered Dilution Strips in a microwell strip holder. One Dilution Well will be required for each standard, (i.e. 0, 2, 5, 20 & 40 ppb) or sample.

2. Place an equal number of Antibody Coated Microwell strips in a microwell strip holder. Return unused microwell strips to the foil pouch with the desiccant packet and reseal pouch with tape.
3. Measure the required amount of Conjugate from the greencapped bottle (~240 µL/well or 2 mL/strip) and place in a separate container (e.g. reagent boat when using the 8- channel pipettor). Using an 8- channel pipette, dispense 200 µL of Conjugate into each blue/green-bordered Dilution Well.
4. Using a single channel pipettor, add 100 µL of each standard or sample into the appropriate Dilution Well containing 200 µL of Conjugate. Use a fresh pipette tip for each standard or sample.

Note: Make sure the pipette tip has been completely emptied.

5. Using an 8-channel pipettor with fresh tips for each 8-well strip, mix each well by carefully pipetting it up and down 3 times and immediately transfer 100 µL of the contents from each Dilution Well into a corresponding Antibody Coated Microwell.
6. Incubate at room temperature for 10 minutes.

Note: Do not agitate the plate to mix as it may cause well-to-well contamination.

7. Empty the contents of the microwell strips into a waste container. Wash by filling each microwell with distilled or deionized water, and then dumping the water from the microwell strips. Repeat this step 4 times for a total of 5 washes.

Note: Take care not to dislodge the strips from the holder during the wash procedure.

8. Lay several layers of absorbent paper towels on a flat surface and tap microwell strips on towels to expel as much residual water as possible after the fifth wash. Dry the bottom of the microwells with a dry cloth or towel.
9. Measure the required amount of Substrate from the bluecapped bottle (~120 µL/well or 1 mL/strip) and dispense into a separate container (e.g. reagent boat for an 8- channel pipettor). Pipette 100 µL of the Substrate into each microwell strip using an 8-channel pipettor. Incubate at room temperature for 5 minutes.
10. Measure the required amount of Stop Solution from the red-capped bottle (~120 µL/well or 1 mL/strip) and dispense into a separate container (e.g. reagent boat for an 8-channel pipettor). Pipette 100 µL of Stop Solution into each microwell strip using an 8-channel pipettor. The color should change from blue to yellow.

11. Read the strips with the **StatFax 4700** using a 450 nm filter with a differential filter of 630nm. Record OD readings for each microwell. Refers to the Quick Start Guide for StatFax 4700 if needed.

Note: Air bubbles should be eliminated prior to reading strips as they may affect analytical results.

Additional Notes:

Ratio of Conjugate to Standard/Sample should remain at 2:1, but volumes of Conjugate and Standards/Samples can be reduced, e.g. using 100µL and 50µL, respectively. The content to be transferred from dilution well to antibody coated well remains the same as 100 µL. Do not return unused reagents to their original bottles. Carefully keep track of the position of Samples and Standards during the assay. Do not mix the assay microwells by shaking at any time during test.

Interpretation of the Results:

Use the **Romer® Log/Logit AQ OTA_2-40 ppb spreadsheet** to interpret the results. Type the OD values obtained from each standard into the Section I of the spreadsheet to build the calibration curve. Make sure that the linearity coefficient (r²) of the calibration curve is no less than 0.985. Type the sample ID and the OD values obtained from each sample into the Section II of the spreadsheet to calculate the actual ochratoxin concentration.

Decision Making:

- If a sample contains ochratoxin levels higher than the highest standard (>40 ppb), the filtered extract should be further diluted in 70% methanol such that the diluted sample results are in a range of 5 - 40 ppb and reanalyzed to obtain accurate results. The dilution factor must be included when the final result is calculated.
- If a sample contains ochratoxin levels below the **limit of quantification (LOQ)** of 2 ppb, then assign a LOQ/2 (in this case would be 1 ppb) for the purpose of calculating the averages.
- If a sample contains ochratoxin levels below the **limit of detection (LOD)** of 1.9 ppb, then assign a value of zero (0) for the purpose of calculating the averages.

Note: An OD value of less than 0.5 absorbance units for 0ppb standard may indicate deterioration of reagents.



PHL Innovation Lab Afghanistan	
Title: Sample Preparation and Test Procedures for T2	
Commodity: Wheat	
Test: T2 ASSAY 20/500 (COKAQ6000/COKAQ6048)	
Written by: <u>Luis Sabillón</u> Effective date: <u>05/11/2015</u>	Edited by: <u>Andréia Bianchini</u> Version: <u>1</u>

Sample Preparation:

1. Obtain a representative sample of wheat flour (or wheat grain) using the Sampling Procedure Protocol.

Note 1: From field samples, if wheat is already ground take a portion of the sample and evaluate the particle size by passing through a 20-mesh screen. If 95% of the sample passes through the sieve, the sample is ready for analysis. If not, the whole sample should be milled using a Romer Series II® Mill.

Note 2: From field samples, if wheat grain is obtained grind the whole sample using a Romer Series II® Mill so that 95 % will pass through a 20-mesh screen, then thoroughly mix the subsample portion.

2. When samples are ready for analysis, ensure that the whole sample is thoroughly mixed.
3. Weigh out 20 g of ground sample into a clean jar that can be tightly sealed, in duplicate.

Note: All remaining sample not used for analysis must be discarded according to the protocol Disposal of Samples.

Note: From this step forward the test needs to be done separate for each extract.

4. Add 100 mL of 70 % methanol and seal jar.

Note: Samples should be extracted in a ratio of 1:5 (w:v) of sample to extraction solution respectively.

5. Blend for 3 minutes at medium speed.
6. Allow sample to settle, then filter the top layer of extract through a Whatman #1 filter and collect the filtrate.
7. Measure the pH of the extract. If needed, adjust pH value in the range of 6.0-8.0 using 1M NaOH or 1M Acetic acid.

Note: Commodity extracts should have a pH of 6.0-8.0. Excessive alkaline or acidic conditions may affect the test results and should be adjusted before testing.

8. The sample is ready for testing without further preparation.

Test Procedure:

Note: All reagents and kit components must be at room temperature 18-30°C (64-86°F) before use. It is recommended that an 8-channel pipettor be used to perform the assay. No more than 48 samples and standards total (6 test strips) should be run in one experiment when using an 8-channel pipettor. If an 8-channel pipettor is not used (i.e. using only single channel pipettes), it is recommended that no more than a total of 16 samples and standards (2 test strips) be run in any one experiment.

1. Place the appropriate number of green bordered Dilution Wells in a microwell strip holder. One dilution well will be required for each standard (0, 20, 50, 150, 500 ppb) or sample.

2. Place an equal number of Antibody Coated Microwell strips in a microwell strip holder. Return unused microwell strips to the foil pouch with the desiccant packet and reseal pouch.
3. Measure the required amount of Conjugate from the green-capped bottle (~240 µl/well or 2 ml/strip) and place in a separate container (e.g. reagent boat when using the 8-channel pipettor). Using an 8-channel pipette, dispense 200 µL of Conjugate into each green-bordered Dilution Well.
4. Using a single channel pipettor, add 100 µL of each standard or sample into the appropriate Dilution Well containing 200 µL of Conjugate. Use a fresh pipette tip for each standard or sample.

Note: Make sure the pipette tip has been completely emptied.

5. Using the 8-channel pipettor with fresh tips for each 8-well strip, mix each well by carefully pipetting it up and down 3 times and immediately transfer 100 µl of contents from each Dilution Well into a corresponding Antibody Coated Microwell.
6. Incubate at room temperature for 10 minutes.

Note: Do not agitate the plate to mix as it may cause well-to-well contamination.

7. Empty the contents of the microwell strips into a waste container. Wash by filling each microwell with distilled or deionized water, and then dumping the water from the microwell strips. Repeat this step 4 times for a total of 5 washes.

Note: Take care not to dislodge the strips from the holder during the wash procedure.

8. Lay several layers of absorbent paper towels on a flat surface and tap microwell strips on towels to expel as much residual water as possible after the fifth wash. Dry the bottom of the microwells with a dry cloth or towel.
9. Measure the required amount of Substrate from the blue-capped bottle (~120 µL/well or 1mL/strip) and dispense into a separate container (e.g. reagent boat for an 8-channel pipettor). Pipette 100 µL of the Substrate into each microwell strip using an 8-channel pipettor.
10. Incubate at room temperature for 5 minutes.
11. Measure the required amount of Stop Solution from the red-capped bottle (~120 µL/well or 1 mL/strip) and dispense into a separate container (e.g. reagent boat for an 8-channel pipettor). Pipette 100 µL of Stop Solution into each microwell strip using an 8-channel pipettor. The color should change from blue to yellow.
12. Read the strips with the **StatFax 4700** using a 450 nm filter with a 630nm differential filter. Record OD readings for each microwell. Refers to the Quick Start Guide for StatFax 4700 if needed.

Note: Air bubbles should be eliminated prior to reading strips as they may affect analytical results.

Additional Notes:

Ratio of Conjugate to Standard/Sample should remain at 2:1, but volumes of Conjugate and Standards/Samples can be reduced, e.g. using 100µL and 50µL, respectively. The content to be transferred from dilution well to antibody coated well remains the same as 100 µL. Do not return unused reagents to their original bottles. Carefully keep track of the position of Samples and Standards during the assay. Do not mix the assay microwells by shaking at any time during test.

Interpretation of the Results:

Use the **Romer® Log/Logit AQ T2_20-500 ppb spreadsheet** to interpret the results. Type the OD values obtained from each standard into the Section I of the spreadsheet to build the calibration curve. Make sure that the linearity coefficient (r^2) of the calibration curve is no less than 0.985. Type the sample ID and the OD values obtained from each sample into the Section II of the spreadsheet to calculate the actual T2 concentration.

Decision Making:

- Samples containing T2 greater than 500 ppb should be further diluted using deionized or distilled water such that the diluted sample results are in the range of 20-500 ppb and reanalyzed to obtain accurate result. The dilution factor must be included when the final result is calculated.
- If a sample contains T2 levels below the **limit of quantification (LOQ)** of 20 ppb, then assign a LOQ/2 (in this case would be 10 ppb) for the purpose of calculating the averages.
- If a sample contains T2 levels below the **limit of detection (LOD)** of 10 ppb, then assign a value of zero (0) for the purpose of calculating the averages.

Note: An OD value of less than 0.5 absorbance units for 0ppb standard may indicate deterioration of reagents.



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Afghanistan

TITLE: Procedure for Decontaminating and Disposing Materials used During Mycotoxin Analysis

Written by: Luis Sabillón
Effective date: 05/11/2015

Edited by: Andréia Bianchini
Version: 1

PURPOSE:

1. To describe the procedure for properly disposing of organic solvents and decontaminating every material used after working with mycotoxins.

PROCEDURES: DISPOSING OF ORGANIC SOLVENTS

1. After extracting the mycotoxin of interest, separate the liquid from the solid portion by filtration.
2. For the extraction procedures involving organic solvents, collect waste chemicals (i.e., methanol) in individual, leak proof, sealed containers. The chemicals must be compatible with container material (e.g. acids must not be placed in a metal container).
3. For the extraction procedures involving water, collect waste water in individual, leak proof, sealed containers.
4. All containers must be clearly identified and labeled with the proper chemical name(s) of the substance(s) at the start of collection.
3. After collection, discard the waste according to the Afghanistan regulations.

PROCEDURES: DECONTAMINATION AND/OR DISPOSING OF SOLID MATERIAL

All contaminated material should be treated as soon as possible. This includes glassware, culturing material, sample residues, etc.

1. After filtration, decontaminate the extraction residues and ground sample materials in 50% bleach for at least 30 minutes. Then, drain it through cheese cloth and discard it in garbage plastic bags.
2. Decontaminate the workspace and any other material used during the extraction procedure as follows:
 - 2.1 Glassware:** The preferred decontamination procedure is to soak in at least 10% bleach solution for 30-60 minutes. Glassware should **never** be left in bleach solution any longer than overnight, as etching can result. Decontaminated glassware should be rinsed with tap water and then washed in the conventional manner.
 - 2.2 Pipettes:** They are decontaminated by placing **tip down** in a pipette can containing 10% bleach. Pipettes should then be rinsed clear of bleach, dipped in nitric acid cleaning solution, rinsed and then washed by conventional methods.

2.3 Metal and plastic utensils (e.g. blender blades, spatulas, foam stoppers, vial caps, etc.): They should be immersed in a bleach solution for only 3-5 minutes. Soaking longer causes rusting and deterioration.

2.4 Culture slants: They should be filled to 1-2 inches from the top with 50% bleach while agar is still warm and molten, and allowed to stand for 30-60 minutes. Strain and discard.

2.5 Work areas and equipment: should be protected from toxin spills, as much as possible, by using plastic liners. The work area should be wiped down with a 10% bleach solution when finished, as should be any pens, pencils, and light equipment used.

Extra sample not ground: Retain these samples for the duration of the project. Once all samples have been processed and data evaluated then combine these retain samples and discard them according to the protocol used to dispose samples in the field (*Protocol – Disposal of Samples*).

PROCEDURES: DECONTAMINATION OF SPILLS

Any spill should be treated as soon as possible according to the following procedure:

1. Treat any spill and any paper towels used with 100% bleach before discarding.
2. Place paper towels over bench top spills and then cover the towels with bleach.
3. Make sure that the entire spill area is treated with bleach. Larger spills or spills that are on the floor or other unprotected surfaces should be surround and covered with an absorbent material (paper towels, vermiculite or other material from a spill kit) then treated with full strength household bleach.
4. Carefully pick up the treated material with a scoop provided in the spill kit and place in a plastic bag.
5. Dispose of the material in the trash.
6. Treat cleaned spill area again by covering with paper towels and treating a second time with full strength bleach.
7. After 5-10 minutes pick up bleach soaked paper towels and place in a plastic bag and dispose of the towels in the trash.
8. Wash the spill area with soapy water.



PHL Innovation Lab
Afghanistan

TITLE: Disposal of Samples

Written by: Luis Sabillon
Effective Date: 05/11/2015

Edited by: Andréia Bianchini
Version: 1

PURPOSE:

1. To describe the procedure for disposing the leftover samples after having taken the representative sample for mycotoxin analysis.

PROCEDURES:

After taking a representative sample(s) (wheat flour, nuts, raisins), discard the remaining sample(s) by following the next steps:

1. Collect the remaining sample(s) in a plastic container.
2. Discard the sample(s) in a hole with dimensions that triple the volume of sample.

Note: The “advisor” must choose a place far away from the participating community where the sample was collected to make the hole.

3. After placing the sample(s), fill the hole with soil to cover it in its entirety.



PHL Innovation Lab
Afghanistan

TITLE: Standard Operating Procedures for Collection, Handling and Storage of Data

Written by: Luis Sabillon

Edited by: Andréia Bianchini

Effective Date: 06/29/2015

Version: 1

PURPOSE:

- To define data management procedures for the analysis of mycotoxins in the Afghanistan's food value chains.

SCOPE:

- This procedure applies to all staff involved in data collection and/or data management for mycotoxin analysis in the Afghanistan's food value chains.

RESPONSABILITIES:

- All site staff members delegated by the Project Manager to collect, record, review, and/or analyze study data are responsible for understanding and following this SOP.
- The Project Manager is responsible for training study staff to collect and manage study data in accordance with this SOP, and for day-to-day oversight of staff involved in data collection and management.

PROCEDURES:

The following procedures are followed to ensure the integrity and expedient retrieval of all data and materials that document activities throughout the study, and those used to record observations and data made regarding the analysis of mycotoxins in the Afghanistan's food value chains.

1. Sample Collection

1.1 Sample Information

The data management process involves handling information collected during the sampling procedure using a standardized form, normally referred to as **Individual Sample Information Form (ISIF)**. Staff members involved in sample collection are responsible for properly collect and transcribe the information onto the ISIF forms. The information collected during sampling include sample identification number, sample description, date of sample collection, sample origin, sample location, type of storage and storage period. The ISIF form must be filled out for each individual sample collected and placed on the corresponding sample bag for proper identification.

1.2 Sampling Control Log - Field

In addition to the ISIF form, the staff members involved in sample collection must also fill out the **Sampling Control Log Field Form (SCLFF)** to keep track of the information provided in the ISIF forms. The information recorded in this form include sample identification number, sample description, sample origin, responsible for shipping, among others. The staff members must keep this form with them all the time.

2. Sample Processing

2.1 Sampling Control Log - Lab

The data management process also involves handling information collected during the receiving of the sample at the lab using a standardized form, normally referred to as **Sampling Control Log Lab Form (SCLLF)**. Once the samples arrive at the lab, the SCLLF must be filled out for each individual sample received. This information is gathered from the SCLFF form located in the sample bag.

When the SCLFF form has been received by the delegated individual, the form should be date stamped and reviewed for any missing data, incomplete fields or data outside normal ranges. If any discrepancies are raised at this stage, these must be clarified with the Project Manager on-site and any queries recorded. Any amendments made on the SCLFF form should be initialed and dated by the Project Manager.

The staff members involved in sample processing at the lab are responsible for properly collect and transcribe this information onto the SCLLF forms. A laboratory identification number is assigned to each individual sample using this form; therefore, an individual sample will have two identification numbers assigned to it.

2.2 Elisa Control Log

The results obtained in the analysis of a particular mycotoxin in each sample must be properly transcribe onto the **Elisa Control Log Form (ECLF)**. The information recorded in this form include laboratory sample identification number, extraction number, sample type, mycotoxin, pH, OD value, among others. The staff members involved in sample processing at the lab are responsible for properly transcribing this information.

2.3 Data Worksheets

Use the mycotoxin-specific **Romer Log/Logit spreadsheet** to interpret the results obtained in the analysis of a particular mycotoxin. The staff members involved in mycotoxin analysis are responsible for properly transcribing the OD values obtained from each standard into the Section I of the spreadsheet to build the calibration curve, to properly type the sample identification and the OD values obtained from each sample into the Section II of the spreadsheet, and to transcribe the pH value, preparation method and dilution for each sample into the Section III to calculate the actual mycotoxin concentration.

3. Data Storage and Protection

All the information collected in the data collection forms must be captured into an electronic record. The Project Manager is responsible for developing an appropriate electronic database to store all data gathered throughout the study. Electronic records must be email to collaborators at the University of Nebraska-Lincoln (UNL) as soon as possible after each form has been filled out completely. UNL staff members will review each electronic record received and routinely issues Quality Control (QC) reports listing queries related to data accuracy, completeness, and consistency. Upon receipt of each UNL QC Report, the project manager must review each QC note and address the QC by amending the appropriate concern/form and re-sending the amended electronic record to UNL staff members.

Electronic records must be kept in a secure drive. Access should be restricted to authorized personnel only and regular backups taken. Backups should be stored securely in a different location from the original data, and checked regularly to ensure that they are working effectively.

Note:

- Small portable media devices should not be used as the primary storage location for data and should not be used for extensive periods.
- All files must be stored on-site in locking cabinets in areas with limited access.
- Any amendments made on the data collection forms should be initialed and dated by the Project Manager.

Appendix VI – Miscellaneous reports

VI.1 Assessment of Ministry of Commerce and Industry fruit and nut export laboratories

VI.2 MAIL Plant Protection Department Capacity Assessment

VI.3 Quality Control Capacity in Afghanistan

VI.4 Progress report – June 2015

VI.5 Progress report – August 2015

VI.6 Mycotoxin Project Fact Sheet



Post-Harvest Loss Innovation Lab Summary of Information on the MoCL/Raisin and Other Dried Fruit Export Promotion Institute Laboratories



Post-Harvest Loss Innovation Lab Summary of Information on the MoCL/Raisin and Other Dried Fruit Export Promotion Institute Laboratories

This Report is the Summary of the Following Reports and Interviews:

“Grain Post-Harvest Training, Storage, and Milling in Afghanistan,” RAMP/ Grain Industry Alliance International (GIAI), Under Chemonics International Inc. Monthly Report-February 2006.

“Stimulating Domestic & Export Market Development Proposal,” Grain Industry Alliance International, March 2007

“Report on Microbiological Training on Analysis of Pathogens in Raisins, Dried Fruits and Nuts.” Moqamuddin Siraj, Funded By: RAMP, GIA, and DWC. November 21-26, 2005.

Data collected by Grain Industry Alliance International (GIAI), 2005 – 2007.

“Request from Raisin and Other Dried Fruit Export Promotion Institute.” MoCI. November 2006

Interview with Raymond Briscoe, Executive Director, DCA-Dutch Committee for Afghanistan, Kabul, Afghanistan. March 2013.

Interview with Greg Cullen, Afghanistan National Horticulture Development Organization (ANHDO), April 2013

Interview with Hershel Weeks, Roots of Peace, March – June 2013.

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ACKNOWLEDGEMENTS:

Lou Faro and Andres Judah of RAMP/USAID/Chemonics

Hirat Fruit Growers

Steven Kovach Co Writer of: "Cold Storage Enterprise Project Design" (CNFA)

These are the author's views expressed in this publication do not necessarily reflect the views of any other agency.

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ACRONYMS

AIB	American Institute of Baking
ANDS	Afghanistan National Development Strategy
ANPP	Afghanistan National Priority Program
ANSA	Afghan National Standards Authority
ANSF	Afghanistan National Strategic Framework
DAIL(s)	Directorate of Agriculture, Irrigation and Livestock
E. coli	Escherichia coli
FAS	Foreign Agriculture Service
GAIN	Global Agricultural Information Network
GAP	Good Agricultural Practices
GIRoA	Government of the Islamic Republic of Afghanistan
GMP	Good Manufacturing Practices
GPS	Global Positioning System
GIAI	Grain Industry Alliance International
HCC	Hepatocellular carcinoma
HPLC	High Performance Liquid Chromatography
KSU	Kansas State University
MAIL	Ministry of Agriculture, Irrigation and Livestock
MoCI	Ministry of Commerce and Industry
MoPH	Ministry of Public Health
MT	Metric Ton
MY	Marketing Year
NADF	National Agricultural Development Framework
OAG	Office of Agriculture
PHL	Post-Harvest Loss
RAMP	Rebuilding Agricultural Markets Program
UNDP	United Nations Development Programme

USAID	United States Agency for International Development
USD	United States Dollars
USDA	United States Department of Agriculture
WFP	UN-World Food Program
WHO	UN-World Health Organization

INTRODUCTION

“Fruit crops are important to some (Afghan) farmers as a cash crop; dried fruits and nuts were once the primary national export. The quality and quantity of Afghan production has declined greatly since the late 1970s. Although established markets have been lost, the potential for sales to the Gulf, Pakistan and India are worth exploring and could be significant.”¹ “Increased dried fruit and nut exports alone are envisaged as reaching \$1 billion annually by 2017.”² Although a major market barrier for Afghan dried fruit and nuts to receive a grade A or premium rating has been the ability to meet international phytosanitary standards. Therefore, in 2005, USAID/RAMP/Chemonics determined there was a need to establish two Ministry of Commerce and Industry (MoCI) dry fruit and nut export certification laboratories in Kabul and Kandahar. The export certification laboratories at the MoCI were determined by the Government of the Islamic Republic of Afghanistan (GIROA) through legislation. GIROA determined MAIL would oversee meat food safety and plant pathology. MoPH had oversight of fortification and imported wheat and flour products. USAID/RAMP/Chemonics requested Grain Industry Alliance International (GIAI) [a collaborative organization of Kansas State University (KSU) and American Institute of Baking (AIB)] to implement establishment of the MOCI laboratories and to conduct training of staff.



Figure 1 Kandahar MoCI Dried Fruit & Nut Lab

“Before the onset of war (1977), horticulture (dried fruits and nuts) represented a substantial portion (Dried fruit represent 18%, 12% for fresh fruit, and 11% for nuts) of Afghanistan’s export income. The Afghan dried fruit and nut sectors has been neglected during the last 25 years. Development of the global horticultural industry has experienced expedient growth since 1977. However, Afghanistan has not benefited from recent innovations in this selection, technological innovations, or marketing. Afghanistan horticulture has been uncompetitive.”³

Afghanistan fresh, dried fruits, and nuts have a significant role in the economy and foreign trade sector. Afghanistan is situated in a temperate region and has a broad variety of fruit production. Grapes are the major cultivated fruit species in more than 15 provinces, representing 48 percent of the country. Apples are cultivated in Wardak and four other provinces. Pomegranates of valuable native varieties are commonly grown in Kandahar, Helmand, Balkh, Nimroz, and Kapisa Provinces. Mulberries are grown in many provinces especially Badghis. Nangarhar is the sole province where farmers grow subtropical trees.

¹ “Afghanistan Natural Resources And Agriculture Sector Comprehensive Needs Assessment – ADB”, January 2002, Pg 138.

² “Afghanistan’s Ministry of Agriculture and Irrigation Master Plan”, MAI, May 2006, Sec 3 – Page 3.

³ “Emergency Horticulture and Livestock Project”, World Bank, May 1, 2006, pg. 8.

Afghanistan's highest quality dried fruits should be processed in sanitary conditions, using dehydration equipment in a hygienic environment. Afghanistan Research Council has been working since 2003 on training of the use dehydration trays from California. Local wood trays and woven plastic sheets have been used as drying surfaces for improved raisin dehydration and produced the highest quality raisins and facilitate the processing of raisins. Moqamuddin Sirij, while employed as a Food Scientist with USAID/RAMP has conducted an aflatoxin survey that indicates a high level of cross contamination occurs in the traditional drying process (Kishmish Khana). Moqamuddin has recommended alternative drying processes and sanitation steps.

Afghan raisins are famous in the global markets and have many potential customers, yet the food safety and sanitation of export raisins needs to meet international phytosanitary standards. Therefore, to achieve economic benefit, growth of exports in the country, to promote, and develop Afghan dried fruits and nuts; it is extremely important to establish a food quality control system and policies based on accepted international standards. This is an area that Afghanistan clearly lags behind other dried fruit and nut exporting countries. The major barrier for development of food quality control systems are a lack of education of Afghans in science and technology. Only a minute number of Afghans understand or have had experience with microbiology, using a microscope, and understand the spread of pathogens.

FOOD HAZARDS IN AFGHANISTAN

Afghanistan health status is one of the poorest in the world. Life expectancy at birth is estimated to be only 45 years for males and 47 years for females. According to surveys conducted by UNICEF, infant, child and maternal mortality rates are some of the highest in the world. Much of the morbidity and mortality is from preventable communicable diseases due to consumption of unhygienic and contaminated food. Chronic malnutrition and the lack of safe food contribute to chronic diseases. This coupled with poor access to health services and poor water sanitation, can lock household members in a vicious cycle of malnutrition and disease, contributing to high mortality rates. Health services and safe water are, at best, in short supply. Afghanistan has extreme weather conditions and outbreaks of major diseases, such as respiratory infections etc. The combination of food insecurity, poor health, and poor sanitation practices has a disastrous impact on the Afghan population.

There are various hazards associated with food that can and do result in injury and harm to human health. The UN – World Health Organization (WHO) estimated that 1.2 million Afghans die each year of food and water borne pathogens. This is due to microbiological hazards and lack of food safety policies, and practices in the country. The issue of food safety and sanitation is part of various ministries [Ministry of Agriculture, Irrigation, & Livestock (MAIL); Ministry of Public Health (MoPH); and Ministry of Commerce & Industry (MoCI)].

Mostly the hazards associated with imported products and commodities (fruits and vegetables) produced in other countries can potentially have biological, chemical, or physical contamination that can cause an adverse health effect. Physical hazards are more easily identified while chemical and biological hazards are far more difficult to understand because of the complexities of interactions between hazards and human biochemistry; and the absence of scientific research, data, and published paper in Afghanistan. Every day thousands of food and food products (wheat flour, vegetable oil, fruits, fruit juices, vegetable,

mineral water, etc.) are imported from Pakistan, Iran, former Soviet States, and Gulf countries. Unfortunately, no agency requires food quality certification on imported goods and neither government laboratory nor private sector institute tests products at the border prior to entry into the Afghanistan.

The food industry has been struggling with food hazards and Hazard Analysis Critical Control Point (HACCP) system for the past decade. The problem is HACCP cannot work unless Good Manufacturing Practices (GMPs) and Good Agricultural Practices (GAPs) are fully functional. Training programs are desperately needed which would teach participants how to integrate the GMP and GAP programs with HACCP in order to implement a world class food safety system.



**Figure 2 Kabul MoCI Dried Fruit & Nut
Lab (2006)**

OBJECTIVE

MAIN OBJECTIVE: The project was intended to support Afghanistan and its relevant institutions in improving the food safety and security status of Afghan fruit producers. The project assisted Afghan traders through a food quality control system by establishing internationally certified laboratories. It worked on capacity building of relevant institutions at central and provincial levels in order to improve food safety, sanitation, and quality standards.

THE SECONDARY OBJECTIVE: The project covered technical assistance and facilitated an integrated approach to improving the food quality, technical training, organized food safety related workshops, and increase the effectiveness of food safety steering committee activities [which had representatives from MAIL-Food Quality Control Department, MoPH, WHO, FAO, and Afghan National Standards Authority (ANSA)⁴]. These quality control laboratories worked for the regular testing of food production in the country and used for import and export food safety verification.

Specific Objectives of the project:

- Conduct internationally accepted physical, chemical, and microbiological testing of Afghan dried and fresh fruits, and vegetable quality, as a means of assessing whether a product has physically, chemically, and microbiologically hazardous. Also, to determine the commodity's recent origin in terms of spoilage and disease born contamination. Microbiological controls were successfully applied to products, to protect public health.

⁴ Afghan National Standards Authority (ANSA), <<http://ansa.gov.af/en/>>

- To evaluate the importance of physical, chemical microbiological contamination in food especially those originating from plant.
- To work on national, regional and international standards, guidelines/legislation and requirements concerning food contamination in fruit exports.

METHOD OF APPROACH TO INTRODUCTION OF KABUL AND KANDAHAR QUALITY ASSURANCE LABORATORIES

The MoCI/Raisin and Other Dried Fruit Export Promotion Institute Laboratories project was based at the KSU/GIAI/AIB Kabul office, with close collaboration with MoCI, MAIL, and the UN. KSU/GIAI/AIB worked in close collaboration with provincial departments and the MAIL Food Quality Control Department.



**Figure 3 Lab Training USAID/RAMP/KSU/GIAI/AIB
remodeled space, installed, equipped,
and Moqamuddin Sirij trained the technicians
for the dry fruit and nut labs in Kabul and Kandahar (2006)**

Dried fruit and nuts are highly susceptible to contamination and thus the percentage of health disorders in Afghanistan has increased day by day, thus it will be better to establish food quality control system to take samples properly and test for food safety at the Afghan border. The establishment of a well-equipped

fresh & dried fruit quality testing laboratories, and providing capacity building services as deemed essential.

LOCATION OF FRUIT TESTING LABORATORIES

The majority of Afghan dried and fresh fruit are grown in the center, north, south, and south east side of Afghanistan. To provide the best service, KSU/GIAI/AIB determined to establish fruit and nut testing laboratories in two important areas of the country where dried fruits and nuts were being exported to many countries throughout the world. Thus KSU/GIAI/AIB installed two dried fruit and nut quality laboratories, one in **Kabul with the Raisin and Other Dried Fruit Export Promotion Institute** and the other with a sub office of the **Raisin and Other Dried Fruit Export Promotion Institute in Kandahar**.

LABORATORY LAYOUT

Layout and design of these laboratories, furniture, and equipment placement was considered on the basis of space available, sampling flow, utility requirements and distribution, special requirements for hazardous sample handling, storage, sterilization, sample retention, and office/clerical/supervisory office space. Mobile or field self-contained testing units were also considered and planned. There were two separate sections in these labs, one for sample preparation, the other for sample analysis, and results interpretation. The equipment installed and chemical reagents are in the following tables:

Table 1

Aflatoxin Determination Equipments						
Inventory #	Description	Quantity	made in	Serial No	Vendor	Date of transfer to Kabul MoC RODFEP - Kandahar Lab
USAID/RAMP00027	Fluorimeter	1	USA	A1617	VICAM	3/25/05
	Disposable Funnel	500	USA		VICAM	3/25/05
	Disposable Pipette	500	USA		VICAM	3/25/05
	Disposable Beaker	500	USA		VICAM	3/25/05
	Grad. Cylinder 50 ml	1	USA		VICAM	3/25/05
	Grad. Cylinder 25ml	1	USA		VICAM	3/25/05
	Vicam Curvet rack	1	USA		VICAM	3/25/05
	Fluted Filter Paper	600	USA		VICAM	3/25/05
	Filter Funnel 65mm	500	USA		VICAM	3/25/05
	Mixer / Blender	1	USA		VICAM	3/25/05
	Afla Test Developer	20	USA		VICAM	3/25/05
	Vicam Wash Bottle	1	USA		VICAM	3/25/05
	Vicam Developer Pipette	1	USA		VICAM	3/25/05
	Vicam Waste Beaker	20	USA		VICAM	3/25/05
USAID/RAMP0028	Afla Standard	3	USA	33020	VICAM	3/25/05
USAID/RAMP0029	Top Loading Balance	1	USA	Sc2000	VICAM	3/25/05
	Filter Paper 24cm	500	USA		VICAM	3/25/05
	Micro Fiber Filter Paper	500	USA		VICAM	3/25/05
	Vicam Pipette	1	USA		VICAM	3/25/05
USAIDRAMP0030	Blender	1	USA	40507	VICAM	3/25/05
	Afla Test Imm Column	500	USA		VICAM	3/25/05
USAIDRAMP0031	Single Position Pump	1	USA	GI500	VICAM	3/25/05
	Vicam Adapter	1	USA		VICAM	3/25/05
	Blender Jar	3	USA		VICAM	3/25/05
	Vicam Curvet	750	USA		VICAM	3/25/05

Table 2

Analytical/Microbiological Equipment						
Inventory #	Description	Quantity	made in	Serial No	Vendor	Date of transfer to Kabul MoC RODFEP - Kandahar Lab
USAIDRAMP0032	Stereoscope	1	India	Seco	Narang	4/10/05
USAIDRAMP0033	Oven 160-42	1	India	Seco	Narang	4/10/05
USAIDRAMP0034	Hot Plate	1	India	Seco	Narang	4/10/05
USAIDRAMP0035	Balance(0.1gram)	1	India	Seco	Narang	4/10/05
	Lab Apron	6	India		Narang	4/10/05
	Tripod Ring	6	India		Narang	4/10/05
	Thermometer	6	India		Narang	4/10/05
USAIDRAMP0036	Autoclave	1	India	Seco	Narang	4/10/05
	Filter Funnel 45mm	6	India		Narang	4/10/05
	Filter Funnel 56mm	6	India		Narang	4/10/05
	Filter Funnel 90mm	6	India		Narang	4/10/05
	Crucible Size 0	12	India		Narang	4/10/05
	Crucible Size 1	12	India		Narang	4/10/05
	Crucible Size 3	12	India		Narang	4/10/05
	Crucible Size D	12	India		Narang	4/10/05
	Crucible Size ICI	12	India		Narang	4/10/05
	Crucible Size KCI	12	India		Narang	4/10/05
	500ml Wash Bottle	3	India		Narang	4/10/05
	Culture Tube 20x150	36	India		Narang	4/10/05
	Culture Tube 16x120	36	India		Narang	4/10/05
	Culture Tube 13x100	24	India		Narang	4/10/05
	Culture Tube 12x75	24	India		Narang	4/10/05
	Culture Tube 16x125	24	India		Narang	4/10/05
	Culture Tube 20x150s	12	India		Narang	4/10/05
	Culture Tube13x100S	12	India		Narang	4/10/05
	Pipette PP865	24	India		Narang	4/10/05
	Flask Pyrex FK100	12	India		Narang	4/10/05
	Centrifuge Brush	6	India		Narang	4/10/05
	Bottle Brush	6	India		Narang	4/10/05
	Burette Brush	12	India		Narang	4/10/05
	Spatulas	6	India		Narang	4/10/05
	Vacuum Pump	5	India		Narang	4/10/05
	Cylinder	4	India		Narang	4/10/05
	Volumetric Flask	12	India		Narang	4/10/05
USAIDRAMP0037	Conductivity Meter	1	India	MD621E	Narang	4/10/05
USAIDRAMP0038	Inf Moisture Balance	1	India	Seco	Narang	4/10/05

Analytical/Microbiological Equipment						
USAIDRAMP0039	Dip Ph Sensor Pj820	1	India	HI 98127	Narang	4/10/05
	Drainage Rack Dy150	2	India		Narang	4/10/05
USAIDRAMP0040	Mag. Stirrer/Hot Plate	1	India	SB162-3	Narang	4/10/05
	Pipette.01ml	6	India		Narang	4/10/05
Nonexpendible	Pipette 1ml	6	India		Narang	4/10/05
	Pipette 10ml	6	India		Narang	4/10/05
	Safety Pipette Filler	6	India		Narang	4/10/05
	Pipette Filler Automatic	4	India		Narang	4/10/05
	Burette Bw105	12	India		Narang	4/10/05
	Pipptor-100ml Cap.	4	India		Narang	4/10/05
	Fisher Tongs	1	India		Narang	4/10/05
	Tube Rack Sm 395	2	India		Narang	4/10/05
	Fuel Burner By400	6	India		Narang	4/10/05
USAIDRAMP0041	Microscope Olympic	1	China	4J02600	Asia Sci	4/10/05
USAIDRAMP0042	Furnace Digital	1		HD485	Asia Sci	4/10/05
	Aspirator Large	1	Germany		Asia Sci	4/10/05
	Aspirator Small	1	Germany		Asia Sci	4/10/05
	Dispenser Large	1	Germany		Asia Sci	4/10/05
	Dispenser Small	1	Germany		Asia Sci	4/10/05
USAIDRAMP0043	Water Still WI460	1	USA		Asia Sci	4/10/05
	Hand Sucking Pumps	3	Pakistan		Asia Sci	4/10/05
	Desiccators De 200	1	Pakistan		Asia Sci	4/10/05
USAIDRAMP0044	Centrifuge 320	1	Taiwan	410947	Asia Sci	4/10/05
	Asbestos Pad	1	China		Asia Sci	4/10/05
	Burner By 400	2	Pakistan		Asia Sci	4/10/05
USAIDRAMP0045	Colorimeter Series	1		NV201	Asia Sci	4/10/05
USAIDRAMP0046	Ph Meter	1	Romania	373593	Asia Sci	4/10/05
USAIDRAMP0047	Refractor Meter Indust.	3			Asia Sci	4/10/05
	Hot Plate	1			Asia Sci	4/10/05
USAIDRAMP0048	Water Both	1		600590	Asia Sci	4/10/05
USAIDRAMP0049	Top Mixer	1		Js1094	Asia Sci	4/10/05
	Pipette Filler				Asia Sci	4/10/05
	Hand Refractometer	3			Asia Sci	4/10/05
	Disposable Filter	150			Asia Sci	4/10/05
	Apron	6			Asia Sci	4/10/05
	Stirrer	2			Asia Sci	4/10/05
	50ml Pyrex Beaker	6	Pakistan		Asia Sci	4/10/05
	100ml Pyrex Beaker	6	Pakistan		Asia Sci	4/10/05
	250ml Pyrex Beaker	6	Pakistan		Asia Sci	4/10/05
	500ml Pyrex Beaker	66	Pakistan		Asia Sci	4/10/05
	1000ml Pyrex Beaker	6	Pakistan		Asia Sci	4/10/05
	3000ml Pyrex Beaker	3	Pakistan		Asia Sci	4/10/05
	Solution Bottles	12	Pakistan		Asia Sci	4/10/05
	500ml Reagent Bottle	24	Pakistan		Asia Sci	4/10/05
	G. Cylinder 25ml	6	Pakistan		Asia Sci	4/10/05
	G. Cylinder 100ml	6	Pakistan		Asia Sci	4/10/05
	G. Cylinder 250ml	6	Pakistan		Asia Sci	4/10/05
	G. Cylinder 1000ml	3	Pakistan		Asia Sci	4/10/05
	G. Flask 100ml	6	Pakistan		Asia Sci	4/10/05
	G. Flask 500ml	6	Pakistan		Asia Sci	4/10/05
	G. Flask 1000ml	3	Pakistan		Asia Sci	4/10/05
	Gloves	200	China		Asia Sci	4/10/05
USAIDRAMP0050	Incubator	1	China		Asia Sci	4/10/05
USAIDRAMP0051	Digital Colony Counter	1	China		Asia Sci	4/10/05
USAIDRAMP0052	Vacuum Pump	1	China		Asia Sci	4/10/05
	Filter Paper	3box	China		Asia Sci	4/10/05
	Surgical Gloves	36	China		Asia Sci	4/10/05
	Magnifier	3	China		Asia Sci	4/10/05
	Mask	50	China		Asia Sci	4/10/05
	spectrophotometer	1	China		Asia Sci	4/10/05

Table 3

Chemical Reagents					
Inventory #	Description	Quantity	made in	Serial No	Transferred
Sodium Chloride	2Kg	India		Narang	5/10/05
Na ₂ HPO ₄	1Kg	India		Narang	5/10/05
KH ₂ PO	1Kg	India		Narang	5/10/05
KCl	1kg	India		Narang	5/10/05
HCl	3lit	India		Narang	5/10/05
Iodine Resublimed	0.50Kg	India		Narang	5/10/05
Acetonitrile	3lit	India		Narang	5/10/05
Zinc Acetate	1Kg	India		Narang	5/10/05
Aluminum Chloride	1Kg	India		Narang	5/10/05
Sucrose	1Kg	India		Narang	5/10/05
EDTA Diammonium Salt	1Kg	India		Narang	5/10/05
NaOH Solution	2lit	India		Narang	5/10/05
Potassium Iodide	0.50kg	India		Narang	5/10/05
Nitric Acid	2lit	India		Narang	5/10/05
NaOH Tablets	2kg	India		Narang	5/10/05
KmNO ₄	1kg	India		Narang	5/10/05
Buffer pH 7 & 14	0.5lit	India		Narang	5/10/05
Methyl Blue	200 gram	India		Narang	5/10/05
Methyl Red	200 gram	India		Narang	5/10/05
Methanol	8 liter	India		Narang	5/10/05

LABORATORY SERVICES

MoCI/Raisin and Other Dried Fruit Export Promotion Institute Dried Fruits and Nuts Analytical Laboratories offered a comprehensive service to ensure dried fruits and nuts safety, nutritional value, and product quality to the processing industries. The Kabul and Kandahar MoCI/Raisin and Other Dried Fruit Export Promotion Institute Dried Fruits and Nuts Analytical Laboratories provided service for the detection of aflatoxin, microbiological analysis, chemical analysis, bacterial identification, grit and sand testing (in Raisin), insect detection, spoilage organism enumeration, sulfur dioxide analysis, fat and oil testing, minerals, and physical testing (grading).

Following were the main quality testing services conducted in these laboratories.

- Physical Analysis
- Analytical Testing (Moisture contents, Brix and Acidity, Grit)
- Microbiological Testing (Plate count Method)
- [Rapid Fluorometer Detection](#) of aflatoxin
- Sulfur Dioxide Detection
- Results interpretation

The Kabul and Kandahar MoCI/Raisin and Other Dried Fruit Export Promotion Institute Dried Fruits and Nuts Analytical Laboratories provided support services including sample collection, registration, and reporting of results.

QUALITY CONTROL CERTIFICATE

Afghan fruit and nut international food safety certification programs play an important role, to clearly demonstrate that the dried fruits and nuts industry takes seriously its responsibility to minimize exposure to all hazards and to provide low risk products.

Aflatoxin

Countries where Afghan dried fruit were exported have been divided into five groups based on their importing policies and regulation. Classification of the following countries of the world countries were according to the EU and WHO regulation and detectible limits of aflatoxin in dried fruits and nuts. Aflatoxin contamination of pistachios, almonds, and raisins are undoubtedly a serious problem in Afghanistan because Afghanistan has not adopted Good Agricultural Practices (GAP). Contamination can occur in crops in the field, at harvest, during post-harvest operations, and in storage. There were several comparative studies have been conducted and results of these studies were presented to Ministry of Commerce, Raisin and Other Dried Fruit Export Promotion Institute staff, and traders.

Conclusion

The installed quality control laboratories at Kabul and Kandahar MoCI/Raisin and Other Dried Fruit Export Promotion Institute Dried Fruits and Nuts Analytical Laboratories worked to issue quality control certificates for the export of dried fruits and nuts during 2003, 2004, 2005. After 2006 support from USAID/RAMP/KSU/GIAI/AIB, there was an inability to acquire the chemical reagents (particularly methanol). The certification was based on fees only and no quantitative data was being collected on aflatoxins. The levels of export were estimated in the following table.

Table 4

No	Quantity of dried fruits exported MT	Year
1	22,437	2003
2	34,414	2004
3	38,539	2005
4	13,200	Within 4 month of 2006

LABORATORY TRAINING PROGRAMS OF USAID/RAMP/KSU/GIAI/AIB

The Objective of the training was:

“To build the capacity and skill of Raisin and Other Dried Fruit Export Promotion Institute staff by providing Microbiology reference services, to improve the standard of dried fruits and nuts quality control laboratory, and promote dried fruits and nuts marketing of Afghanistan”.

During this training the participants were trained in the characteristics of pathogenic and non-pathogenic bacteria, and microbiological testing procedures for dried fruits and nuts. They also worked on microbial grouping that are associated with food and water; theory and practical; and enumeration; identification; and isolation of E.coli, Coliforms, Salmonella, and Listeria in raisins, almonds, pistachios, apricots, and cashews. They worked on mycotoxin and its significance in food and dried fruits. The group worked on HPLC techniques to determined aflatoxin B1, B2, G1 and M1.

Aflatoxin Training Program

Training about awareness of aflatoxin and testing procedure in dried fruit and nuts was organized in RAMP Office on 11th to 13th of January, 2005. In this training 23 people were invited from Raisin And Other Dried Fruit Export Promotion Institute, MAIL, and Faculty of Agriculture, and Faculty of Science Kabul University.

The main objective of this training was determination of aflatoxin in food, with specific reference to practical testing of aflatoxin in dried fruits and nuts. In this training course the participants learned the issues and problems associated with dried fruits, participants worked on improving analytical practices, and determined the level of aflatoxin in almond, pistachios, apricot, and walnut.



Figure 4 Aflatoxin Training

Training on introduction to Aflatoxin Fruits Microbiology

KSU/GIAI/AIB organized a training course for the Kandahar lab staff at the MoCI/Raisin and Other Dried Fruit Export Promotion Institute Laboratory. Technicians from Kandahar worked on quality assurance tests of aflatoxin, microbiological, and chemical analysis of dried fruits and nuts including bacterial identification, Grit and sand testing (in dried fruits), insect detection, spoilage organism enumeration, and sulfur dioxide analysis.

KSU/GIAI/AIB developed a protocol manual regarding aflatoxin, sampling methods, and comparative studies of raisin, almond and pistachios. The aflatoxin protocol manual was developed to introduce the

concepts of aflatoxin, how to take sample, sampling methods, and determination of aflatoxin through flourometric method, and preventative measures. This manual was helpful for the quality control staff of the MoCI/Raisin and Other Dried Fruit Export Promotion Institute Laboratories, and some major growers and processor, to understand the safe drying and storage of dried fruits and nuts. This manual was also translated in Dari language for easy understanding by the local traders.

Training for Determination of Pathogenic Bacteria determination

To improve the capacity of MoCI/Raisin and Other Dried Fruit Export Promotion Institute Laboratories' technical staff, 10 day training was organized by KSU/GIAI/AIB entitled "Determination of Pathogenic Bacteria in Dried Fruits and Nuts" at the Central Food Technological Research Institute (CFTRI) and the Nodal Codex Microbiological Laboratories (the National Food Safety Laboratories), Mysore-Bangalore, India.

Participants of the Training:

1. Moqamuddin Siraj, (RAMP's Senior Food Technologist)
2. Faridoon, (Laboratory Technicians at Raisin and Other Dried Fruit Export Promotion Institute).
3. Khuja Abdul Rehman, (Laboratory Technicians at Raisin and Other Dried Fruit Export Promotion Institute).
5. Abdul Wase Azizi, (Laboratory Technicians at Raisin and Other Dried Fruit Export Promotion Institute).

Dr. J. S. Sandhu conducted a HACCP and GMP training. The participants complete a HACCP Plan. The participants participated in a lab on food sampling and sample preparation.

Dr. J. S. Sandhu stated that there were three recommended methods of culturing Microorganisms in dried fruits and Nuts.

- Plate culture methods (PC)
- Most Probable Number (MPN)
- Membrane Filtration (MF)

The Afghan participants all received the related information including a microbiology manual that was provided by Dr. Prema Viswanth, the manager of the microbiology laboratory in CFTRI. The pathogenic bacteria in the enumeration session were Escherichia coli (E. coli), Listeria monocytogenes, Enterobacteriaceae, and Salmonella sp.

Dr. Varadraj taught the participants how to test for sulfur dioxide. Also, he taught the basics on the HPLC. MoCI/Raisin and Other Dried Fruit Export Promotion Institute Laboratory's technical staff felt the training was very beneficial.

The Major Barrier for the Effectiveness of an Afghan Food Safety Program is the Need for Human Resources

One food safety expert need be too hired from aboard while other staff will be Afghan professionals. These will be including!

- Food safety expert
- Food Quality Specialist
- Admin/Accounting Manager
- Food Experts
- Hygiene and sanitation officers
- Consultants

The Evaluation of the Needs for Afghanistan is that for Food Scientists

A core group of “Afghan trained Food Scientists” are needed to develop the export marketing potential, address food safety, and sustain food safety in Afghanistan. This core group of food safety experts would be composed of 10 MS and PHDs in various disciplines of food safety. Without the internationally trained food safety special, Afghanistan lacks the human capacity to implement a food safety program.

SWOT ANALYSIS

<p>Strengths</p> <ul style="list-style-type: none"> • Support by MoCI, MAIL, MoPH, fruit and nut traders, and donors to improve food safety in Afghanistan. • Fruit and nut traders want certification so they can enter more profitable markets. 	<p>Weakness</p> <ul style="list-style-type: none"> • Difficult to acquire reagents and lab supplies in Afghanistan. • Raisin and Other Dried Fruit Export Promotion Institute is issuing certification based on fees only without quantitative testing. • The results of the weak Afghan education system results in a general lack of understanding of food microbiology and food safety.
<p>Opportunities</p> <ul style="list-style-type: none"> • Create a core group of Food Safety Specialist by educating 10 MS and PHDs in food science. 	<p>Threats</p> <ul style="list-style-type: none"> • Even though Afghans have the credentials on paper to conduct food safety analysis but they often lack a basic understanding of laboratory protocol and food microbiology.

Capacity Assessment of Plant Protection and Quarantine Department

Plant Protection and Quarantine Department (PPQD) of the Ministry of Agriculture, Irrigation and Livestock (MAIL)

Background information: The plant protection and quarantine department was established in 1319 (1940) as independent directorate for Pest control. During 1954 the directorate is named “Locus Control” directorate. During 1961 it’s affiliated with Ministry of Agriculture and named as Plant Protection and Quarantine Department (PPQD). Initially, the Plant Protection and Quarantine Department (PPQD) had limited activities but gradually expanded, equipped with required equipment and staffing. During September 1985 to December 1986 the Plant Protection and Quarantine Department (PPQD) was technically supported by the FAO/UNDP through project named “Strengthening Plant Protection Services”. The projects had key role for increasing the capacities of the plant protection services included the completion of laboratories for entomology, plant pathology, nematology and pesticide analysis, as well as of the quarantine station at Kabul customs. But during the civil war 1979 to 2000 these labs were totally destroyed and looted by armed groups.

The Plant Protection and Quarantine Department (PPQD) labs were rehabilitated for the second time in 2004 under the FAO project “Follow-up of emergency locust control in northern Afghanistan and renovation of Laboratory of the Plant Protection and Quarantine Department ,Ministry of Agriculture and Animal Husbandry. Through this project FAO implemented developing an integrated plant protection program in consultation with RAMP/USAID, based on the substantial experiences gained from previous years. Without the support of the project, Plant Protection and Quarantine Department (PPQD) would have had less capacity to respond promptly to the past and eventual swarm invasions.

Major achievements of PPQD: During the course of ten years 2004-2014, the Plant Protection and Quarantine Department (PPQD) had significant contribution in terms of supporting farmers, students, released agriculture related articles/publications and conducted some mini-research studies. During 10 years (2004-2014) the Plant Protection and Quarantine Department provided recommendations (prescriptions) to 175,672 farmers after testing/checking various samples of plant diseases, nematodes and pests. Nearly 900 students from Faculty of Agriculture, Kabul University and Institute of Agriculture have been trained in plant protection and quarantine related issues. An average, 350 students are getting plant protection related technical training annually.

More than 60 article regarding plant protection have been published in “Karhana” local magazine of Ministry of Agriculture, Irrigation and Livestock (MAIL). Through support of various donors and NGOs, research studies have been undertaking on Melon fly *Mypardale Pardline* , *wilt in melon* and identification of *brown tile moth* in melon. Three additional laboratories have been established in Kabul, Nangarhar and Herta province. A biological control lab and an insect museum have been established in Plant Protection and Quarantine Department (PPQD) lab in Badam Bagh area of Kabul city.

Objective of the assessment: The overall goal of plant protection laboratory and professional staff capacity evaluation is to i) know the level technical and analytical expertise of staff working in Plant Protection and Quarantine laboratories ii) check and confirm the availability of analytical equipment and materials in the PPQD laboratories. These efforts will further help in designing capacity building related

training for determination of mycotoxin and understanding basic causes of postharvest losses due to mycotoxin.

Key activities of PPQD laboratories is, conducting laboratory analysis, undertaking laboratory experiments, providing training on lab testing, analysis and identification of insect pests. The PPQD is also supporting the identifying farmer problems and providing the required assessment for solving their problems.

Key findings:

Plant protection and quality control issues become increasingly important in Afghanistan. For food producers, processors, manufacturers, and traders needs significantly plant protection as well as food quality control system and regulatory environment.

Staff, skills and training: Staff capacity evaluation was done through bilateral meetings with technical staff working in Plant Protection and Quarantine (PPQ) laboratories. During the meetings, the senior management of Plant Protection and Quarantine (PPQ) Mr. Ghorbandi (Head), Mr. Tahir (Deputy) and staff members' plant protection and Quarantine were informed about the overview and objective of a rapid assessment and causes of prevalence of mycotoxins in wheat, dried fruits and high value horticulture crops. Detail information was provided about the mycology, species of fungus and major causes of mycotoxin that affecting the staple food and fruits and its negative effects on health and export of cereals, fresh and dried fruits.

Detailed face to face meetings were held with Mr. Abdul Ghafoor Baburi head of Diagnostic labs, Mr. Mohammad Nasir Ebrahimkhail Insect identification specialist, Mr. Jamaluddin Stankzai biological control assistant, Mr. Abdul Wasi Hakimi seed pathologist and Mrs. Patoni Azizi biological control assistant. During our meetings, it's appeared that one of the key challenge after rehabilitation of these labs was impeded by the limited capabilities of plant protection and quarantine laboratory staff and their abilities use the available apparatus and equipment for determination of plant pathogens mainly bacteria, virus and nematode but their technical capacity was enhanced when in-service courses on "basic sciences and in laboratory techniques" were provided by FAO, USAID and RAMP during 2005-2010.

Plant Protection and Quarantine Department (PPQD) has 23 technical staff. Out of 23, 3 are Master degree holders who have 5-16 years of working experience with MAIL plus 5-10 year laboratory experience and 13 staff are Bachelor degree holders have 5-15 years relevant field experience including 2-5 year laboratory experience. All technical staff have basic knowledge of relevant physical and chemical tests of cereals, Horticulture crops, fruits and other food products. They are conducting routine Physical and chemical tests in laboratories of Plant Protection and Quarantine Department. Physical tests includes the identification of insect pest, identification of nematodes, observation of pathogen affected part of plants under microscope and determination of plant pathogens and relevant plant diseases. Bacteria related tests are includes culture of bacterial affected samples of cereals and horticulture crops.

The staff of Plant Protection and Quarantine Department (PPQD) have very little information about standards, protocol preparation, supervision and interpretation of test results reporting and checking of microbiology, nematology results. They also have very little familiarity with test and calibration

procedures and awareness of limitations of these procedures. Moreover, managerial capabilities, equipment calibration requirements, familiarity with method validation, proficiency testing and QC checks is highly required.

Sampling and testing in PPQD:

Sampling procedure: There is no proper sampling collecting procedure/guidelines and available in the PPQD. Majority of the farmers do not know the accurate sample collecting/taking process and also unaware that to whom they should refer/send the sample of plant protection related cases. In case of plant pathogen, insect pest and nematodes, farmers are collecting samples and sending to relevant nearby district agriculture office, where they are seeking their support in terms of identification of relevant plant protection issues and get guidance to deal with. Sometime the staff of PPQD collecting the sample when they are on field mission. Thus, the method of sample collection and sending further for analytical test is with non-technical way. Moreover, there is no sample handling and shipment facilities available in the PPQD. There is need of Sample Collection Manual and Laboratory Procedures Manual to proceed analytical testing of the samples.

Field visits: The staff of Plant Protection and Quarantine Department (PPQD) are conducting occasional field visits to province and district from where PPQD is getting plant protection related reports and relevant concerns. In case of large scale plant protection issues e.g. Melon fly attack in Kunduz and other part northern provinces, PPQD is sending a group of technical staff to observe the situation and collect samples collection, survey the area and organizing meeting with farmers through support of agriculture extension workers in relevant province.

In some cases donors or NGOs who are implementing plant protection related activities are officially informing and inviting the PPQD staff to attend the workshop or capacity development training at field level.

Sample testing: An average, staff of Plant Protection and Quarantine Department (PPQD) is conducting 2,500 physical, microbiological and chemical test annually. Usually samples of plant pathology related diseases (barks, leaves, roots and other part of the plant) are sending by farmers to district based MAIL staff based in provincial center. After physical check (identification of symptoms) some samples are resending back to farmers with recommendations. In some cases, the MAIL staff based in provincial center is sending more complicated and unclear sample further to Kabul for further technical testing and research.

In Kabul, the staff Plant Protection and Quarantine Department (PPQD) registering samples with sample specifications (plant/fruits name, types, date of sending and registration). After registration, the sample is sending further to relevant lab for physical, chemical and microbiological testing. The staff of Plant Protection and Quarantine Department (PPQD) are conducting tests based on the initial information received from the farmers. The staff of relevant laboratory is testing the samples physically and sometime chemically when necessary. The technical staff of PPQD testing the sample and preparing technical report about presence of pathogen, nematodes and its infection. The technical staff of PPQD sending back the sample received and send back the outcomes of their tests and providing recommendation about the application of chemical or mechanical practices to control the relevant plant protection issues.

There is no duplicate or replicate sample for testing to provides accessible internal comparisons and contributes to the validation of the analytic phase. These sources may be previously tested samples, samples of known constituents, and already reported proficiency testing samples. This tool assesses the analytic phase only.

Record keeping: After testing of field sample, the staff of Plant Protection and Quarantine Department (PPQD) is keeping the a sample for one month to double check in case of any complain from the farmers or field staff of the MAIL based in the province. There is very limited capacity of sample rack or sample keeping space in Plant Protection and Quarantine Department (PPQD).

Knowledge about Mycology and Mycotoxins. The technical working in Plant Protection and Quarantine Department have basic knowledge and information about Mycology and Mycotoxins as well as its causes of during post-harvest of agriculture products. While they have limited information about determination of mycotoxin through ELISA, flourimetric and other mycotoxin determination methods. Majority of them also interested to know proper sampling method of food stuff (cereals, fruits and vegetable) in big size warehouse or small scale storage facilities.

Limited research work: The Plant Protection and Quarantine Department is providing training opportunities for students from faculty of agriculture and Institute of agriculture located in Kabul. There no research related activities ongoing in these laboratories.

Quarantine work: According to head of Plant Protection and Quarantine laboratories, PPQD assigned staff in international airports in Kabul, Kandahar, Mazar and Herat airports. These staff are doing follow and tracking the exporting and importing food items. They have no testing facilities in the airport but sending samples to PPQD for physical and analytical testing.



Microbiological laboratory- sterilization and culture

Facilities available in laboratories: Prior to check the availability of laboratory equipment, we decided to check wither the present equipment are functioning or not. It also discussed that wither all laboratory staff understand how the equipment works, how to operate it, safety considerations in using the equipment and how to clean and put away the equipment. There are five small labs in Plant Protection and Quarantine Department (PPQD). Culture/Microbiology lab, Nematology, Plant Pathology, Entomology and Seed health laboratories. The Nematology, Entomology and Seed health labs are very basic. These laboratories are limited with availability of few microscopes, mish, sieves and magnifications lenses etc. Culture/Microbiology lab is comparatively better equipped with required

apparatus/equipment. Culture/Microbiology lab have various types of microscopes including digital microscope, incubator, blinder, Autoclave, Oven, Laminar flow, Centrifuge, Electric balance, Microscopes, Glassware (cylinders, beakers, flasks, funnels, petri dish, pipettes) and media e.g. Agar-agar and dextrose.

Equipment available in the PPQD labs

No	Name of equipment	Quantity	Status
1	BINOCULAR MICROSCOPE	36	Functioning
2	COMPOUND MICROSCOPE	6	Functioning
3	MICROSCOPE ATTACHED WITH CAMERA	1	Functioning
4	AUTOCLAVE FOR STERILIZATION	1	Functioning
5	LAMINAR AIR FLOW CHAMBER	1	Functioning
6	CENTRIFUGE	1	Functioning
7	OVEN	1	Functioning
8	ELECTRIC BALANCE	2	Functioning
9	REFRIGERATOR	1	Functioning
10	INOCULATION NEEDLE	1	Functioning
11	SLIDES	24	Functioning
12	COVER SLIDES	24	Functioning
13	PETRI DISH	24	Functioning
14	TEST TUBE SMALL	24	Functioning
15	TEST TUBE MIDIMUM	24	Functioning
16	TEST TUBE LARGE	24	Functioning
17	MAGNIFYING LENS	6	Functioning
18	SLANDER DIFF	6	Functioning
19	FLASK	6	Functioning
20	BEAKER	12	Functioning
21	Protective cloths (complete set)	12	Functioning

There is no distillation machine available in Plant Protection and Quarantine Department (PPQD). The professional staff in these laboratories are using tap water for conducting their routine tests. The Plant Protection and Quarantine Department (PPQD) has limited number of lab refrigerators but have no freezer. Moreover, there is no consumables, disposable gloves and face masks, disposable pipettes and accessories available in the laboratory. City power is still a problem in Kabul mostly in winter and extreme weather situations. An electrical generator was requested by laboratory staff.

In Addition to ELISA kit- following equipment needed for the Mycotoxin Laboratory

No	Name of equipment	Quantity
1	Water distillation machines	1
2	Lab freezer or refrigerator	1
3	Disposable gloves	10 dozen

4	Disposable pipettes	60 (different size)
5	Face masks	10 dozen
6	Sample keeping racks	2
7	Electricity/power generator	1



PPQD museum with insect samples

Recommendations:

1. Food quality testing is a multi-disciplinary activity covering a number of aspects such as food science, microbiology, analytical chemistry, plant pathology, veterinary science, etc. where various government partners involved in country level including MAIL, MoCI, MoPH and research institutions, agricultural institutions, farming community, trade associations, non-governmental organizations (NGOs), consumers etc. There is desperate needs of clear role and responsibility of each entity to take timely action and avoid duplication of efforts.
2. Lack of certification systems is one of the major bottleneck for export promotion of Afghan dried and fresh fruits. The Plant Protection and Quarantine departments have their network and presence in Kabul international airport but there is no proper sampling testing facilities in the airport. The technical staff of Plant Protection and Quarantine laboratory is testing the sample only through physical appearance and some simple physical test. More attention required to work on better sampling and quality testing of food items in airports and other border location of Afghanistan.
3. Technical staff of Plant Protection and Quarantine laboratories have limited knowledge and information about standards, protocol preparation, supervision, interpretation of test results reporting and checking results. They also have very little familiarity with food quality testing including mycotoxin testing, calibration procedures and awareness of limitations of these procedures. There is high needs of well equipped (in terms of technical staffing and equipment) laboratories with clear objectives, including well designed plan of action with role clarity provided for different players and strong networking of the organizations at country level. Moreover, managerial capabilities, equipment calibration requirements, familiarity with method validation, proficiency testing and QC checks is highly required.

4. More regular and systematic basis training and capacity building efforts required to keep the lab staff updated and have awareness on the scenario for inspection, sample collecting and analytical testing in the country as well as the latest testing techniques, risk analysis and record keeping, auditing techniques, etc.
5. Water distillation machine, freezer and an electric generator are highly needed to run the mycotoxin testing in Plant Protection and Quarantine Department. Moreover, there is no consumables, disposable gloves and face masks, disposable pipettes and accessories in the laboratories of Plant Protection and Quarantine laboratory.
6. Plant Protection and Quarantine laboratories have some chemical which is enough for routine work of samples testing. The laboratory facing shortage of chemicals (list attached) to uses for training of students from Faculty of Agriculture, University of Kabul and Institute of Agriculture located in Kabul. The head of Plant Protection and Quarantine department recommended to consider the procurement of enlisted chemicals to continue the capacity building and training activities for university/institute students.
7. Awareness and causes of Mycotoxin is vital to help farmers, growers, warehouse owners and transporters of food (cereals, dried and fruits etc.) to take it into consideration, the important steps during post-harvest practices during harvesting/picking, handling, storage and transportation of food and avoid the chance of cross contamination of mycotoxin in agriculture products. It will further contribute provide mycotoxin free food and food products during post harvesting and prior to reaching to local and international market.

Key meeting with following PPQD staff

Name	Job title	Purposes of the meeting
Mr. Gurbandi	Head of Plant Protection and Quarantine Departments	Discussion establishment of Mycology laboratory, training, certifications and maintenance system
Mr. Tahir	Deputy/ admin of Plant Protection and Quarantine	Discussion on technical aspect and management of quality control laboratories
Abdul Ghafoor Baburi	Head of Diagnostic Lab	Discussion on technical aspect and management of quality control laboratories
Mohammad Nasir Ebrahimkhail	Insect pest specialist	Discussion about qualification, experience, skills regarding routine tests and needs of more technical support.
Jamaluddin Stankzai	Insect Ecologist	Discussion about qualification, experience, skills regarding routine tests and needs of more technical support.
Abdul Wasi Hakimi	Seed pathologist	Discussion about qualification, experience, skills regarding routine tests and needs of more technical support.
Patoni Azizi	Biological control assistant	Discussion about qualification, experience, skills regarding routine tests and needs of more technical support.
Ejaz Ahmad	Consultant- Biological control	Discussion about qualification, experience, skills regarding routine tests and needs of more technical support.
Qudratullah Soofizada	Change management specialist	Discussion about qualification, experience, skills regarding routine tests and needs of more technical support.
Mirwais Khogyiani	Change management specialist	Discussion about qualification, experience, skills regarding routine tests and needs of more technical support.

Summary on Quality Control Situation in Afghanistan

By Sirij Moqamuddin

Key findings:

- Available buildings with 20 rooms in Kabul , Mazar-e – sharif, and Kandahar provinces
- 12 staff in Kabul , and no instruments for food quality testing
- quarantine inspectors; at the border points conduct visual inspection
- No food safety testing; conducted on unsafe/ substandard samples.

Current Scenario:

- According to MOU between MAIL and MOPH, MAIL is responsible for testing the quality of raw/ unprocessed agricultural products,
- MAIL assigned testing task to food quality control department (FQCD).
- New MAIL Tahskeel/ Org Chart- FQCD was eliminated and replaced with seed Certification.
- Incorporated food quality control (FQC) in Plant Protection and Quarantine Directorate (PPQD)

MAIL needs for sustainability of the quality testing:

- lab equipment and staff training
- Technical assistance lab efficiency / quality
- Management system and accreditation.

ANDOH / Privet sector assessment and recommendations:

First Section Results

The quality system (QC) in Afghanistan can be described as composed by the following bodies:

- 1.Ministry of Public Health-Food Drug Quality Control Laboratories (MOPH),
- 2.Ministry of Commerce,
- 3.Ministry of Agriculture Irrigation and Livestock
- 4.Department of Quarantine
- 5.ANSA, Afghanistan National Standards Authority
- 6.AICC (Afghanistan Chamber of Commerce and Industries).

A fundamental part of the QC analysis applied to PH are the QC laboratories. Laboratories tests provide an immediate and quantifiable index of the quality of the products in terms of food safety and security.

In general a QC lab has the following roles:

1. Evaluate food safety in terms of:

- Microbiological hazards (e.g. on food or packaging)
- Chemical Hazard: Mycotoxins (e.g. Ochratoxin and Aflatoxin) or Heavy metals Pesticide and other chemical residues, etc.
- Control the Ingredients' composition, additives, Food and Nutrition Information.

Beside the analytical control, the PH's QC Laboratory is also a fundamental part of the National Control System. Its roles are:

- Food Fraud Alerts
- Risk assessment methodology
- Food contamination emergencies
- Custom control on Export/Import products
- Food safety regulations, policy and Standards

Evidently, the QC system cannot be entrusted to one single Minister or Lab.

Private labs should to be included in the QC system. Private laboratories, even if not identified as QC labs, are part of the food control system because all the laboratories results are tools to conduct analytical inspection on different foods, both for private companies and public institutions. If the test is made by private laboratory, the procedure is out of the institutional control. In any case, it represents an occasion for the private companies to understand the level of their food processing and/or of their final product. Such a test does not imply the consequences of possible non conformity revealed by institutional labs. For the above mentioned reason, among the others, ARFVPA represents the most relevant private laboratory in the Afghan QC system. ARFVPA release the Certificate of Quality that represents, as well as the other entire laboratory test, a synthesis of the tested food quality. Moreover such certificate is mandatory for the export. Due to its role, ARFVPA has been deeply investigated during the mission.

The following list represents a non-exhaustive database of the existing laboratories in Kabul, involved in QC:

1. Afghanistan Raisin Fruits and Vegetables Promotion Administration (ARFVPA) or erroneously called EPA (MoC)
2. Food Drug Quality Control Laboratory (MoPH)
3. Ex.QC-Department laboratory (MAIL)
4. Afghanistan National Standards Authority (ANSA)
5. Tabasom raisin factory (Private Laboratory)
6. Pomology Labs

The situation of the QC in Afghanistan is now blocked, and several scenarios are possible. As the MAIL Deputy Minister said: in the next one or two years all the responsibilities for the food QC should pass under the Quarantine Department and Animal Health Department. In this scenario, the ARFVPA laboratory seems to lose part of its importance. However, the current Afghan political situation is not stable, and the MAIL Policy may change in the near future.

Suggested actions and recommendations

The Afghan QC system might be improved through the following Actions:

1. Use the good relation with the Pomology and Biotechnology Labs, in association with external Laboratories (Universities and private ones)
 - a. As explained during the PHPD Post-Harvest seminar, SO3 could implement a pilot shipment to EU using secured and standard procedures for each step of the shipment, checking each single step with ELISA tests. The pilot delivery (e.g. raisin and almonds) will be monitored and checked by SO3 staff.
 - b. SO3 could create an Inter-laboratory ring test. Such kind of protocol leads to an improvement of quality of analytical results, and provides clear information of measurement's capabilities of the participating laboratories. This idea was shared with, and received support from, the MAIL Deputy Minister.
2. SO3 project has already created a strong collaboration with Tecna. In the next months this connection should be strengthened to develop a dedicated mycotoxin raisin extraction procedures.
3. SO3 should introduce the principles of ISO 17025 in the existing laboratories involved in the ring test above mentioned. ANSA should also be involved in this Action.
4. SO3 team needs a cycle of internal training on the following issues:
 - HACCP and other hygienic procedures
 - ISO procedures (9001, 22000 and 19011)
5. In view of the information emerged during the meeting with the MAIL Deputy Minister, the MAIL future policy on Quality Certificate and QC system must be ascertained as soon as possible; Such a clarification is necessary to decide which kind of approach should be adopted with ARFVPA. According to which will be the role of ARFVPA,
6. ANHDO, through the HPS, will be in condition to create an internal ISO consultancy service to help its members applying ISO procedures.
7. The QC laboratories survey has been completed according to the Work Order. In the future HPS might help MAIL (and/or other Ministers) in providing a more deeply assessment of the existing laboratories. A detailed list of gaps and possible solutions for each recognized bottleneck will be released, including required equipment, training, and costs.

**Progress Report for the Feed the Future Innovation Lab
for the Reduction of Post-Harvest Loss Afghanistan Project
January 1, 2015 – May 31, 2015**

Submitted by the PHL Innovation Lab Afghanistan Project Team Members:

**Dr. Dirk Maier, PI
Dr. Andreia Bianchini, Co-PI
Dr. Venkat Reddy, Co-PI
Dr. John Leslie, Co-PI
Ms. Deb Frey, Project Coordinator**

June 15, 2015

I. Research Progress Summary

A. Research progress made during the reporting period

The primary research progress to report during this period was a thorough literature review regarding mycotoxin contamination in Afghanistan food products. During this review the Afghanistan mycotoxin research conducted by the United Nations World Food Program (UN-WFP) and USAID Rebuilding Agricultural Markets Program (RAMP) in Afghanistan were included. As a result of limited research and data available on mycotoxin contamination in Afghanistan's food system, the literature review included available data in surrounding countries, i.e., former Soviet Union states, Iran, Pakistan and India. Based on this literature review, the climate prevalent in the country and available epidemiological data related to cancer in Afghanistan's population, the research methodology for this project was further developed and refined.

1. Design Research Methodology and Initiate Capacity Building

1.1 Design a research and sampling methodology based on international best practices

Activities

Based on the background information gathered the PHL Innovation Lab Afghanistan project team determined the mycotoxins of interest for wheat, raisins, and nuts would be:

- Aflatoxin (AFL), which is a mycotoxin produced by *Aspergillus flavus* and *Aspergillus parasiticus* that occurs in grains, raisins, and nuts. High exposure of AFL can result in elevated levels of stunted growth, delayed development, and hepatic cancers.
- Deoxynivalenol (DON) or vomitoxin, which is a toxin produced by *Fusarium* species. This mycotoxin could be present in imported flour and flour products into Afghanistan that have been noted to have an off odor and taste, and appears dark in color.
- Trichothecene (T2/HT2), another mycotoxin produced by *Fusarium* spp. that occurs in grain grown at high elevation. High exposure to T2 causes a reduction in leukocytes which results in bronchial pneumonia and lung bleeding.
- Ochratoxin (OTA), which is a mycotoxin produced by *Aspergillus carbonarius* and *Penicillium verrucosum* that occurs in grains and raisins. High exposure to OTA can result in renal adenomas, renal carcinomas, and neurotoxic effects in the developing brain.

The PHL Innovation Lab Afghanistan project team, prior to inception of field activities, developed the approach and methodology for conducting the assessment based on international best practices and standards. A literature review was done to evaluate the best approach to representatively collect samples in the field and markets of Afghanistan. Additionally, practices regarding sample preparation were also evaluated and tested at the mycotoxin laboratory at University of Nebraska – Lincoln, which is a partner in the PHL Innovation Lab.

Progress

Based on findings, Standard Operation Procedures (SOPs) were (or are in the process of being) prepared in order to detail the steps involved in sample collection and transfer to the MAIL lab in Kabul where analysis will be performed; in sample preparation and analysis; and in data processing, storage and transfer to U.S.-based collaborators. Additionally, SOPs related to decontamination and safety were also developed. The following SOPs have already being completed: **Sampling Procedure Protocol**

- Sampling Control Log
- Sample Preparation and Test Procedures for Aflatoxin: Raisins and Nuts (e.g., pistachios, almonds, walnuts)
- Sample Preparation and Test Procedures for Ochratoxin: Raisins and Nuts (e.g., pistachios, almonds, walnuts)
- Sample Preparation and Test Procedures for Aflatoxin: Wheat
- Sample Preparation and Test Procedures for Deoxynivalenol: Wheat
- Sample Preparation and Test Procedures for Ochratoxin: Wheat
- Sample Preparation and Test Procedures for T2: Wheat
- Quick Start Guide for AgraVision Strip Reader
- Quick Start Guide for StatFax 4700 Microstrip Reader
- ELISA Control Log
- General Laboratory Safety Precautions Concerning Mycotoxin Analysis
- Procedure for Decontaminating and Disposing Materials used During Mycotoxin Analysis
- Disposable of Samples

All completed protocols have been transferred to the USAID Afghanistan mission office for translation into the country's official language.

Challenges

The success of this project greatly depends upon a representative and well executed sample collection, as well as precise mycotoxin analysis in such samples. Given that such a thorough and systematic survey of the magnitude proposed in this project has never been done in the country, the MAIL staff involved little to no experience with sampling and analysis for mycotoxins. Therefore, training will be provided to address this lack of experience and to ensure that the SOPs developed for the project are closely followed.

1.2 Train MAIL field staff in usage of sampling technology

Activities

Central to this investigation will be the use of low-cost, rapid assaying kits that are used to detect mycotoxins. There are several commercial suppliers of these kits (e.g. <http://www.elisa-tek.com/diagnostic-testing-kits/mycotoxins/>). The PHL Innovation Lab Afghanistan project team has selected the most appropriate for the proposed task.

The assessment will be undertaken in key markets and production nodes (e.g., wheat flour depots, packing houses) and will be coordinated in conjunction with MAIL. MAIL staff will be trained by the PHL Innovation Lab Afghanistan project team in all necessary protocols in order to carry sample collection and mycotoxin analysis.

Progress

In order to provide training to the MAIL and DAIL staff in Afghanistan, Deb Frey (Project Coordinator) was trained in all necessary protocols at the UNL mycotoxin laboratory. Deb, after receiving an intensive 3-day training, will serve as the trainer to MAIL and DAIL staff and as manager for all daily operations related to the sample collection and analysis in Afghanistan.

Prior to Deb's arrival in Kabul a series of pre-training videos will be distributed to the appropriate MAIL and DAIL staff. Three weeks of training will be conducted when PHL Innovation Lab Afghanistan project staff arrives in Afghanistan in mid-July. One week will be focused on sampling and the next two weeks will be focused on training for conducting the assays. MAIL staff will be training as a food safety cohort in the following methodologies:

- Sample collection in the field, transfer to Kabul laboratory and preparation for analysis.
- Protocols and materials to conduct AFL AgraQuant® ELISA analysis for wheat, raisins, and nuts.
- Protocols and materials to conduct OTA AgraQuant® ELISA analysis for wheat and raisins.
- Protocols and materials to conduct T2/H2 AgraQuant® ELISA analysis for wheat.
- Protocols and materials to conduct DON AgraStrip analysis for wheat.
- All protocols related to decontamination, safety and disposal of excess samples.

The MAIL laboratory staff will have the opportunity to become proficient in the methodologies related to food safety used in this project. At the end of this training and completion of the sample analysis, the MAIL staff will be able to help establish a food safety system related to mycotoxins in Afghanistan by applying their acquired experience in sample collection and analysis. Ultimately this would lead to an improvement of food safety throughout Afghanistan and its export market potential, especially regarding nuts and raisins. Most importantly, the establishment of such a system could lead to a reduction in observed childhood stunting and developmental delays, as well as liver and kidney cancers.

Challenges

Many MAIL staff have not had the tertiary education in biology, chemistry, math and laboratory procedures that is required to sustain long-term a food safety system par to other international programs. However, the training provided in this project, along with continuing education and training in food safety certainly could address this knowledge and experience gap.

1.3 Establishment of needed infrastructure

Activities

To complement building technical capacity within MAIL, this project will also provide support for equipment and supplies deemed essential by the PHL Innovation Lab Afghanistan project team in order to continue mycotoxin research and detection beyond the life of this project.

Progress

Procurement and purchase of equipment and material determined by the PHL Innovation Lab Afghanistan project team as needed to establish one fully operating mycotoxin laboratory and three other satellite laboratories was accomplished. The main laboratory for mycotoxin analysis will have the capacity to prepare, store and analyze any kind of samples (including samples requiring grinding), while each satellite laboratory would be able to analyze samples that do not require grinding or that were previously ground in the main laboratory. Some required equipment (e.g., water distillation system) will only be located in the main laboratory, but could easily be used to support research and analysis in the satellite locations.

Equipment and material that will be sent to Afghanistan include, but are not limited to: sampling probes, water distillation system, mill, mycotoxin test kit readers, mycotoxin test kits, food processors and blenders, scales,

pH meters, vortexes, pipettes, dispensers, glass jars, test tubes, and consumables (e.g., pipette tips, sampling bags, and wipes).

All equipment and material needed in Afghanistan has been prepared for cargo shipment and is currently under the care of MEBS Global Reach, LC to be sent to Kabul, once all required paperwork has been finished.

Challenges

Challenges related with shipping such as amount of equipment and material needed overseas include ensuring (1) all proper documentation needed to transport and customs clearance is in place; and (2) the safety and integrity of the equipment until its final destination because of conflicts in the area where the project is being executed and extreme conditions in warehouses while awaiting customs clearance. To address these challenges help from MAIL and USAID Afghanistan mission staff has been requested and proven instrumental. Cargo insurance is also being procured and assurance from the shipping company has been sought on safeguarding as best as possible the performance of the test kits by keeping them away from sunlight and for the shortest length possible in warehouses that may not have controlled temperatures.

2. Implement Data Collection and Sample Analysis

2.1 Implement sample collection

Activities

The PHL Innovation Lab Afghanistan project team will work with MAIL staff to sample and assay commodities located in various parts of the country. MAIL staff time and facilities will serve as the GIRoA’s in-kind contribution to this assessment project.

The sampling plan for the entire project was developed based on consumer consumption patterns and export importance of specific commodities. The total number of samples collected for analysis will be 500 to 800; with 30% flour and wheat products, 23% raisins, 18% almonds, 18% pistachios, and 11% walnuts. GPS coordinates will be recorded at every site for follow-up.

Progress

The wheat sampling plan was determined based on the data gathered by USDA – Foreign Agricultural Service – Global Agricultural Information Network (USDA-FAS-GAIN) which indicates that 80% of all wheat and wheat food products is of Afghan origin, 10% is Pakistani flour, 4% is of Kazakhstan origin, 3% is of Uzbekistan origin, and 3% is of Iranian origin. A total of 75% of all flour milled in Afghanistan is milled in the small asiab mills (stone mills) or grist mills (small, portable, roller mills). The wheat samplings were divided according to reported consumption habits and production (Table 1). Wheat sampling will occur in Kabul, Kunduz, Herat, and Mazar-I Sharif.

Table 1: Proposed Wheat / Flour / Wheat Products Sampling Scheme.

Type of Samples	Number of Samples
Asiab and Grist Mill Flour of Afghan Origin	104 - 144
Asiab and Grist Mill Flour of Kazakhstan Origin	5 - 9
Asiab and Grist Mill Flour of Uzbekistan Origin	3 - 6
Purdue Improved Crop Storage (PICS) bags	2 - 4
Two warehouses or storage facilities in Mazar-i-Sharif, Kabul, Kunduz, and Herat, as recommended by grain traders or farmers.	8

Two naan bakeries in each of the four regions	8 - 20
Two – four flour millers in each of the four regions	8 - 20
In Kabul market sampling of Pakistan flour	6 - 12
In Herat market sampling of Iran wheat products	3 - 6
In Kabul market sampling of other flour	3 - 6
TOTAL	150 - 235

Raisins have high potential for export into the international markets. The Afghan raisins have unique flavor and variety. Afghan raisins have a high degree of Brix (fructose levels). Raisin sampling will occur in Kabul, Shamali Plain, Herat, Mazar-i Sharif, and Kandahar. The seven major varieties of raisins will be sampled as illustrated in the table below.

Table 2: Proposed Raisins Sampling Scheme.

Type of Samples	Number of Samples
Medium Quality Round Green Raisin	18 - 27
Medium Quality Long Green Seedless Raisin	18 - 27
High Quality Shundurkhani Raisin (golden-high value)	16 - 26
Medium Quality Red Raisin	12 - 27
Sun dried Shomali Raisin (dark red)	15 - 27
Sun dried Ghazni Raisin (dark red)	15 - 27
Sun dried Tayefe Raisin (Mazar-i-Sharif - rose colored)	15 - 27
TOTAL	110 - 188

Almonds, pistachios and walnuts have high potential for export into the international markets. Afghan nuts have unique flavor and variety. Afghan almond flavonoids are more bioactive than almonds grown in the West. Almonds with these high levels of flavonoids could be niche marketed if these almonds could meet international phytosanitary standards. The major varieties of nuts will be sampled as illustrated in Tables 3-5.

Table 3: Proposed Almond Sampling Scheme.

Type of Samples	Number of Samples
Sattarbai Soft-shell Almonds (Mazar-i-Sharif)	15 - 24
Shokorbai Hard-shell Almonds	15 - 24
Abdul Wahidi Almonds (Mazar-i-Sharif)	15 - 24
Qambari Almonds	15 - 24
Ghorbandi Almonds	15 - 24
Sangaki and Murawaji Almonds (smaller kernels)	15 - 21
TOTAL	90 – 141

Table 4: Proposed Pistachio Sampling Scheme.

Type of Samples	Number of Samples
Korak Pistachios	23 - 38
Pushdara Pistachios	23 - 38
Khandan-e-safid Pistachios	23 - 38
Other varieties of Pistachios	21 - 36
TOTAL	90 – 142

Table 5: Proposed Walnut Sampling Scheme.

Type of Samples	Number of Samples
Zard Walnuts (yellow kernels)	10 - 17
Mazaari Walnuts (yellow kernels)	10 - 17
Takhari Walnuts	10 - 17
Korek Walnuts	10 - 17
Kaghazi Walnuts (paper shelled)	10 - 17
Other varieties of Walnuts	10 - 15
TOTAL	60 - 94

Sampling protocols have been developed for all commodities of interest, as previously mentioned in this progress report. As the samples arrive from the field in the Kabul MAIL laboratory, the assays will be conducted starting with the raisins because these samples would be the most susceptible ones to allow mold growth and toxin production during storage.

Challenges

MAIL and DAIL staff have had limited access to diagnostic laboratories and limited experience with food safety. However, the proposed training that will occur in July in Afghanistan for all involved in this project will address any concerns related to this lack of knowledge and experience.

2.2. Implement sample analysis and data collection

Activities

Based on the literature review, the climate prevalent in the country and available epidemiological data related to cancer in Afghan's population, the mycotoxins of concern in the commodities of interest were defined and the testing methods chosen.

Progress

The following mycotoxin test kits from Romer Labs were chosen for this project:

- AgraStrip for DON to be used on wheat analysis
- AgraQuant for AFL to be used on wheat analysis
- AgraQuant for OTA to be used on wheat analysis
- AgraQuant for T2 to be used on wheat analysis
- AgraQtrip for AFL to be used on the analysis of raisins and nuts
- AgraQuant for OTA to be used on the analysis of raisins and nuts

All chosen kits have been validated by the manufacturer (Romer Labs) for the use on the evaluation of mycotoxin levels in the commodities of interest to this project. Protocols related to the use of the test kits chosen for the project were prepared to standardize the quantification of mycotoxins in the samples.

Challenges

The concepts associated with mycotoxins and sampling are new for MAIL and DAIL staff. Therefore detailed training and close monitoring of activities will be carried out until staff are following the proposed methodology in a precise and safe manner.

3. *Generate Project Reports and Disseminate Findings*

3.1 Generate progress and final report which will summarize findings and propose recommendations for the follow-up actions

Activities

The PHL Innovation Lab Afghanistan project team will generate progress and final reports that will summarize findings and propose recommendations for follow up actions. The final report is to be shared with MAIL, MoCI, MoPH, and WFP.

Progress

This progress reports is the first one submitted.

Challenges

N/A

3.2 Support the organization of an international workshop on the reduction of post-harvest losses outside of Afghanistan.

Activities

The PHL Innovation Lab Afghanistan project team will provide administrative and technical support for organizing an international workshop in support of addressing pre- and post-harvest losses with a special emphasis on Afghanistan. This workshop will take place outside of Afghanistan near the end of this project.

Progress

Nothing to report at this time.

Challenges

N/A

B. Issues or concerns encountered during the reporting period

- Visa Approval for Project Coordinator – The project coordinator (Deb Frey) applied on April 20th, 2015 for a multiple entry visa to Afghanistan and thanks to repeated follow up with contacts in Afghanistan the visa was finally issued the week of May 25. This delayed the travel date of the project coordinator and the planned training of MAIL project personnel to mid-July.
- Shipping of Mycotoxin Testing and Other Supplies – This has proven to be a bigger challenge than initially anticipated and thus took longer than planned. A shipping facilitator was found with the help of USAID which will allow for smooth transition through customs in Afghanistan and transfer to MAIL. Additionally, all materials from Romer Laboratories and assembled at the University of Nebraska are now in-hand and are in the process of being shipped to Afghanistan. Some will be hand-carried by the project coordinator. This delay in shipping, anticipated arrival date and custom clearance of equipment and material was another factor delaying travel of the project coordinator to mid-July.
- Protocols have been finalized and have been submitted to MAIL for translation in preparation of staff training.
- Recent discussions during weekly project conference calls revealed that disbursement of travel funds including per diem for MAIL staff cannot be paid directly by the PHL Innovation Lab project coordinator

while in Afghanistan. The USAID Mission Office is currently exploring alternative options on how these funds can be paid out.

- As a result of project delays, a revised timeline with a proposed no cost extension for the current project through February 28, 2016 is included with this progress report.
- As a result of project delays, additional effort by project collaborators, and changes in anticipated project personnel and partners, a revised budget for the current project through February 28, 2016 is included with this progress report. An initially proposed subcontract to UC Davis has been replaced with a proposed subcontract to Iowa State University. The subcontract amount to University of Nebraska Lincoln has been substantially increased because the project Co-lead PI (Bianchini) and her staff have spent considerably more time and effort on project planning, supplies and materials identification and order preparation, and training of the project coordinator than initially planned. The total budget allocated for this project remains unchanged.
- As a result of two project Co-PIs (Maier, Reddy) relocating from Kansas State University to Iowa State University, the revised budget includes the recommendation to award a subcontract to Iowa State University in order for the existing project team to continue collaborating on the successful execution and completion of this project. The total budget allocated for this project remains unchanged.

C. Data Sharing and Dissemination

No progress to report in this area.

II. Human and Institutional Capacity Development

A. Short-term training

- See activity 1.2 in terms of training MAIL staff to collect samples along the value chains, to receive samples and prepare them for analysis, and to analyze samples for mycotoxin presence with the provided equipment and according to standard laboratory practices.

B. Long-term training

- There are no plans in the current project for long-term training.

C. Institutional capacity development

- See activity 1.3 in terms of equipping a designated mycotoxin laboratory in MAIL for the purpose of providing food safety analysis services to the value chains beyond the life of this current project.

III. Technology Transfer and Scaling Partnerships

- The primary technology transfer will be to MAIL in terms of equipping a mycotoxin testing laboratory and training technical staff to operate this lab according to internationally accepted standards.

IV. Future Work

- Depending on the results of this value chain assessment a follow-up project may be developed and recommended.

**Progress Report for the Feed the Future Innovation Lab
for the Reduction of Post-Harvest Loss Afghanistan Project
January 1, 2015 – August 30, 2015**

Submitted by the PHL Innovation Lab Afghanistan Project Team Members:

**Dr. Dirk Maier, PI
Dr. Andreia Bianchini, Co-PI
Dr. Venkat Reddy, Co-PI
Dr. John Leslie, Co-PI
Ms. Debra Frey, Project Coordinator**

August 30, 2015

ACRONYMS

ANDS	Afghanistan National Development Strategy
ANPP	Afghanistan National Priority Program
ANSF	Afghanistan National Strategic Framework
DAIL(s)	Directorate of Agriculture, Irrigation and Livestock
E. coli	Escherichia coli
ELISA	enzyme-linked immunosorbent assay
FAS	Foreign Agriculture Service
GAIN	Global Agricultural Information Network
GAP	Good Agricultural Practices
GIRoA	Government of the Islamic Republic of Afghanistan
GMP	Good Manufacturing Practices
GPS	Global Positioning System
GIAI	Grain Industry Alliance International
HCC	Hepatocellular carcinoma
HPLC	High Performance Liquid Chromatography
KSU	Kansas State University
MAIL	Ministry of Agriculture, Irrigation and Livestock

MAIL-PPQD	Ministry of Agriculture, Irrigation and Livestock-Plant Protection and Quarantine Directorate
MoCI	Ministry of Commerce and Industry
MoPH	Ministry of Public Health
MSDS	Material safety data sheets
MT	Metric Ton
MY	Marketing Year
NADF	National Agricultural Development Framework
OAG	Office of Agriculture
PHL	Post-Harvest Loss
RAMP	Rebuilding Agricultural Markets Program
SOP	Standard Operational Procedures
UNDP	United Nations Development Programme
USAID	United States Agency for International Development
USD	United States Dollars
USDA	United States Department of Agriculture
UN-WFP	UN-World Food Program
WHO	UN-World Health Organization

I. Research Progress Summary

A. Research progress made during the reporting period

The primary research progress to report during this period was a thorough literature review regarding mycotoxin contamination in Afghanistan food products. During this review the Afghanistan mycotoxin research conducted by the United Nations World Food Program (UN-WFP) and USAID Rebuilding Agricultural Markets Program (RAMP) in Afghanistan were included. As a result of limited research and data available on mycotoxin contamination in Afghanistan's food system, the literature review included available data in surrounding countries, i.e., former Soviet Union states, Iran, Pakistan and India. Based on this literature review, the climate prevalent in the country and available epidemiological data related to cancer in Afghanistan's population, the research methodology for this project was further developed and refined.

Detailed methodology was finalized in March 2015, approved by Ministry of Agriculture, Irrigation and Livestock-Plant Protection and Quarantine Directorate (MAIL-PPQD), and United States Agency for International Development (USAID). Comprehensive Standard Operating Procedures (SOPs) and protocols were developed in May through June 2015. June and July 2015 the SOPs and protocols were translated into Dari and Pashtu by USAID translators. The laboratory shipment arrived at MAIL-PPQD on the 20th of July 14th, 2015 with all laboratory equipment intact.

1. Design Research Methodology and Initiate Capacity Building

1.1 Design a research and sampling methodology based on international best practices

Activities

Based on the background information gathered the PHL Innovation Lab Afghanistan project team determined the mycotoxins of interest for wheat, raisins, and nuts would be:

- Aflatoxin (AFL), which is a mycotoxin, produced by *Aspergillus flavus* and *Aspergillus parasiticus* that occurs in grains, raisins, and nuts. High exposure of AFL can result in elevated levels of stunted growth, delayed development, and hepatic cancers.
- Deoxynivalenol (DON) or vomitoxin, which is a toxin produced by *Fusarium* species. This mycotoxin could be present in imported flour and flour products into Afghanistan that have been noted to have an off odor and taste, and appears dark in color.
- Trichothecene (T2/HT2), another mycotoxin produced by *Fusarium* spp. that occurs in grain grown at high elevation. High exposure to T2 causes a reduction in leukocytes which results in bronchial pneumonia and lung bleeding.
- Ochratoxin (OTA), which is a mycotoxin produced by *Aspergillus carbonarius* and *Penicillium verrucosum* that occurs in grains and raisins. High exposure to OTA can result in renal adenomas, renal carcinomas, and neurotoxic effects in the developing brain.

The PHL Innovation Lab Afghanistan project team, prior to inception of field activities, developed the approach and methodology for conducting the assessment based on international best practices and standards. A literature review was done to evaluate the best approach to representatively collect samples in the field and markets of Afghanistan. Additionally, practices regarding sample preparation were also evaluated and tested at the mycotoxin laboratory at University of Nebraska – Lincoln, which is a partner in the PHL Innovation Lab.

Progress

Based on findings, Standard Operation Procedures (SOPs) were prepared in order to detail the steps involved in sample collection and transfer to the Afghanistan Ministry of Agriculture, Irrigation, and Livestock-Plant Protection & Quarantine Directorate (MAIL-PPQD) lab where analysis will be performed; in sample preparation and analysis; and in data processing, storage and transfer to U.S.-based collaborators. Additionally, SOPs related to decontamination and safety was also developed. The following SOPs have been completed:

- Standard Operation Procedures (SOPs)
- Sampling Procedure Protocol
- Sampling Control Log
- Sample Preparation and Test Procedures for Aflatoxin: Raisins and Nuts (e.g., pistachios, almonds, walnuts)
- Sample Preparation and Test Procedures for Ochratoxin: Raisins and Nuts (e.g., pistachios, almonds, walnuts)
- Sample Preparation and Test Procedures for Aflatoxin: Wheat
- Sample Preparation and Test Procedures for Deoxynivalenol: Wheat
- Sample Preparation and Test Procedures for Ochratoxin: Wheat
- Sample Preparation and Test Procedures for T2: Wheat
- Quick Start Guide for AgraVision Strip Reader
- Quick Start Guide for StatFax 4700 Microstrip Reader
- ELISA Control Log
- General Laboratory Safety Precautions Concerning Mycotoxin Analysis
- Procedure for Decontaminating and Disposing Materials used During Mycotoxin Analysis
- Disposal of Samples

Thanks to USAID Afghanistan Mission Office staff for translation of all protocols. All protocols were translated into Dari and Pashto.

Challenges

The success of this project greatly depends upon a representative and well executed sample collection, as well as precise mycotoxin analysis in such samples. Given that such a thorough and systematic survey of the magnitude proposed in this project has never been done in the country, the MAIL-PPQD staff involved has little to no experience with sampling and analysis for mycotoxins. Therefore, training will be provided to address this lack of experience and to ensure that the SOPs developed for the project are closely followed.

1.2 Train MAIL field staff in usage of sampling technology

Activities

Central to this investigation will be the use of low-cost, rapid assaying kits that are used to detect mycotoxins. There are several commercial suppliers of these kits (e.g. <http://www.elisa-tek.com/diagnostic-testing-kits/mycotoxins/>). The PHL Innovation Lab Afghanistan project team has selected the most appropriate for the proposed task.

The assessment will be undertaken in key markets and production nodes (e.g., wheat flour warehouses, packing houses) and will be coordinated in conjunction with MAIL-PPQD. The MAIL-PPQD and Directorate Agriculture, Irrigation, and Livestock (Provincial DAIL)-PPQD staff will be trained by the PHL Innovation Lab Afghanistan project team in all necessary protocols in order to carry sample collection and mycotoxin analysis.

Progress

Prior to arrival in Kabul, Debra Frey, Project Manager sent a series of pre-training videos to be distributed to the appropriate MAIL and DAIL staff. These videos contained how to **assemble** the distillers, set-up of other equipment, calibration, background information regarding mycotoxins and ELISA. The MAIL and DAIL staff found these videos to be very helpful.

- Sampling training was provided on July 28th and 29th, 2015 to the MAIL-PPQD and DAIL staff in Afghanistan, Debra Frey, Project Manager) trained MAIL-PPQD and DAIL staff on all necessary protocols for sampling developed at the UNL mycotoxin laboratory, including: Sample collection in the field, transfer to Kabul laboratory and preparation for analysis.
- Protocols and materials to conduct AFL AgraQuant® ELISA analysis for wheat, raisins, and nuts.
- Protocols and materials to conduct OTA AgraQuant® ELISA analysis for wheat and raisins.
- Protocols and materials to conduct T2/H2 AgraQuant® ELISA analysis for wheat.
- Protocols and materials to conduct DON AgraStrip analysis for wheat.
- All protocols related to decontamination, safety and disposal of excess samples.



Figure 1: Two brain storming sessions were conducted on how to address food safety in Afghanistan. Support of the Afghanistan Government's adoption of Codex Alimentarius was discussed.

The MAIL-PPQD laboratory staff has the opportunity to become proficient in the methodologies related to food safety used in this project. At the end of this training and completion of the sample analysis, the MAIL-PPQD staff will be able to help establish a food safety system related to mycotoxins in Afghanistan by applying their acquired experience in sample collection and analysis. Ultimately this would lead to an improvement of food safety throughout Afghanistan and its export market potential, especially regarding nuts and raisins. Most importantly, the establishment of such a system could lead to a reduction in observed childhood stunting and developmental delays, as well as liver and kidney cancers. The economic effect would be that Afghanistan high value export crops (nuts and raisins) could be certified as below allowable limits of mycotoxins.

Table 1: List of staffs for the Mycotoxin sample analysis training

#	Name	Designation	Qualification (Level)	Specialization	Phone	Email
PPQD Staff						
1	Kh. Aminuddin	Plant Quarantine General Manager	BSc	Plant Protection	(0)799445671	kwawajaa4o@yahoo.com
2	Jahid Ahadi	Plant Quarantine Inspector at Kabul Custom	BSc	Plant Protection	700275706	jahedahady@gmail.com
3	Mohammad Rafi Rustami	Plant Quarantine Manager in Kabul Airport	BSc	Plant Protection	799241676	mrafirustami@gab.com
4	Abdul Ghafoor Baburi	Head of Diagnostic Lab	BSc	Agronomy	700259618	
5	Aziz Ahmad Sakhri	Microbiology	MSc	Plant Protection	793645354	aziz-sakhiz-02@yahoo.com

6	Assadullah Ansari	Plant Pathology	MSc	Plant Protection	7723787979	a-ansoriaz8@xana.com
7	Patoni Azizi	Biological Control Manager	BSc	Plant Protection	781147169	
8	Wazhma Noorzai	Weed Manager	14th grade	Plant Protection	788811747	
9	Zakira Qadori	Technician	12th grade	Plant Protection	782920214	
10	Mohammad Tahir Habib	Plant Diseases - General Manager	BSc	Plant Protection	799246732	habib.mtaherz90@gmail.com
11	Azim Khan Habib	IPM manager	BSc	Plant Protection	706484101	azimkhanhabibl@gmail.com
12	Hemayatullah	Mycologist	MSc	Plant Pathology	799707423	hemayatullahrahil@yahoo.com
13	Mohamad Naser Ibrahim Khail	Entomology - General Manager	BSc	Plant Pathology	799309151	m.naseribrahimkhail@gmail.com
14	Abdul Razaq Moafaq Moafaq		BSc	Plant Protection	798982592	amafaq-3@gmail.com
15	Eng. Basir Ahmad Tabib			Plant Protection		-
16	Abdul Qadeer Safai			Plant Protection	796151016	-
17	Abdul Wadod Ghorbandi	Director		Plant Protection		-
18	Mr. Qudratullah Soofizada			Agriculture Research Institute of Afgh/MAIL		-
19	Mr. Moh. Iqbal. Karimi			Plant Protection	(0)780357291	-
20	Mr. Zakria Faizi	Lab Tech		Plant Protection		-
DAIL Staff		Department		Province		
21	Abdul Shokar	PPQD		Kunduz	(0)799012216	
22	Abdul Mateen	PPQD		Kunduz	(0)772429081	
23	Najibullah	PPQD		Kunduz	(0)787151052	
24	M. Raqib	PPQD		Parwan	(0)704501710	
25	Sakhi Ahmad	PPQD		Parwan	(0)788504801	
26	Ghulam Darwood	PPQD		Parwan	(0)771630804	
27	Zabihullah	PPQD		Kandahar	(0)706567062	
28	Mohammad Nasin	PPQD		Kandahar	(0)783758965	
29	Ali Ahmad	PPQD		Kandahar	(0)	

					703639866	
30	M. Martaza Mhmoody	PPQD		Herat	(0) 708068141	
31	Eng. Basir Ahmad Tabib	Manager PPQD		Herat	(0) 708463741	
32	Wazir Ahmad Dastmohammdi	Pest & Disease		Herat		
33	Naqibulla	PPQD		Kabul District	(0) 777175701	
34	Alauddin Ansari	PPQD		Bakh	(0) 700510630	
35	Monawarshah Almadi	PPQD		Bakh	(0) 799025029	
36	Sefatullah Fizi	PPQD		Bakh	(0) 799268707	

Challenges

Many MAIL staff has not had the tertiary education in biology, chemistry, math and laboratory procedures that is required to sustain long-term a food safety system par to other international programs. However, the training provided in this project, along with continuing education and training in food safety certainly could address this knowledge and experience gap.

1.3 Training MAIL-staff on sample analysis

Analysis training was conducted from August 15th to August 24th, 2015. Lab overviews were conducted on all protocols, SOPs, material safety data sheets (MSDS), equipment operations, and log procedure.



Figure 2: The MAIL PPQD Staff, Representatives from Ministry of Public Health, Ministry of Commerce, Kabul University, and MAIL Research.



Figure 3: The Analysis Training Group Practiced Pipetting, Calibrating Balance, Calibrating PH Meter, and Extensive Sample Preparation.



Figure 4: Conducting the First Mycotoxin Assays

The training group became knowledgeable about theory of toxicity of any given substance. The group as a whole is showing high comfort levels with the protocols and lab procedure. Two individuals work as a team to decrease error and conduct self-monitoring.

Challenges

The concepts associated with mycotoxins and sampling are new for MAIL-PPQD and DAIL staff. Therefore detailed training and close monitoring of activities were carried out to ensure that all staff involved in the project could follow the proposed methodology in a precise and safe manner. Additionally, during sample analysis project supervisor will continue to monitor staff performance and re-train as needed to ensure safety in the laboratory and quality of the data collected.

1.4 Establishment of needed infrastructure

Activities

To complement building technical capacity within MAIL-PPQD, this project will also provide support for equipment and supplies deemed essential by the PHL Innovation Lab Afghanistan project team in order to continue mycotoxin research and detection beyond the life of this project.

Progress

Procurement, purchase, and shipment of equipment and material determined by the PHL Innovation Lab Afghanistan project team as needed to establish one fully operating mycotoxin laboratory was accomplished. The laboratory established in Kabul (details about location) for mycotoxin analysis will have the capacity to prepare, store and analyze a variety of samples (including samples requiring grinding)]. Additional supporting equipment (i.e. stabilizers and backup batteries) have also been provided.

Equipment and material that was sent to Afghanistan include, but are not limited to: sampling probes, water distillation systems, mill, mycotoxin test kit readers, mycotoxin test kits, food processors and blenders, scales, pH meters, vortexes, pipettes, dispensers, glass jars, test tubes, and consumables (e.g., pipette tips, sampling bags, and wipes).

All equipment and material needed in Afghanistan has been shipped and arrived safely under the care of MEBS Global Reach, LC to Badam Bagh, Kabul, Afghanistan.

Stabilizers and backup batteries have been installed by RMA Group not only for the mycotoxin labs but also for the culture lab and pest lab. This will give two hours of back-up system to conclude any tests being conducted if a power outage should occur. In Figure 7, the red boxes outline the areas occupied by the mycotoxin lab and the other areas are the other PPQD labs.

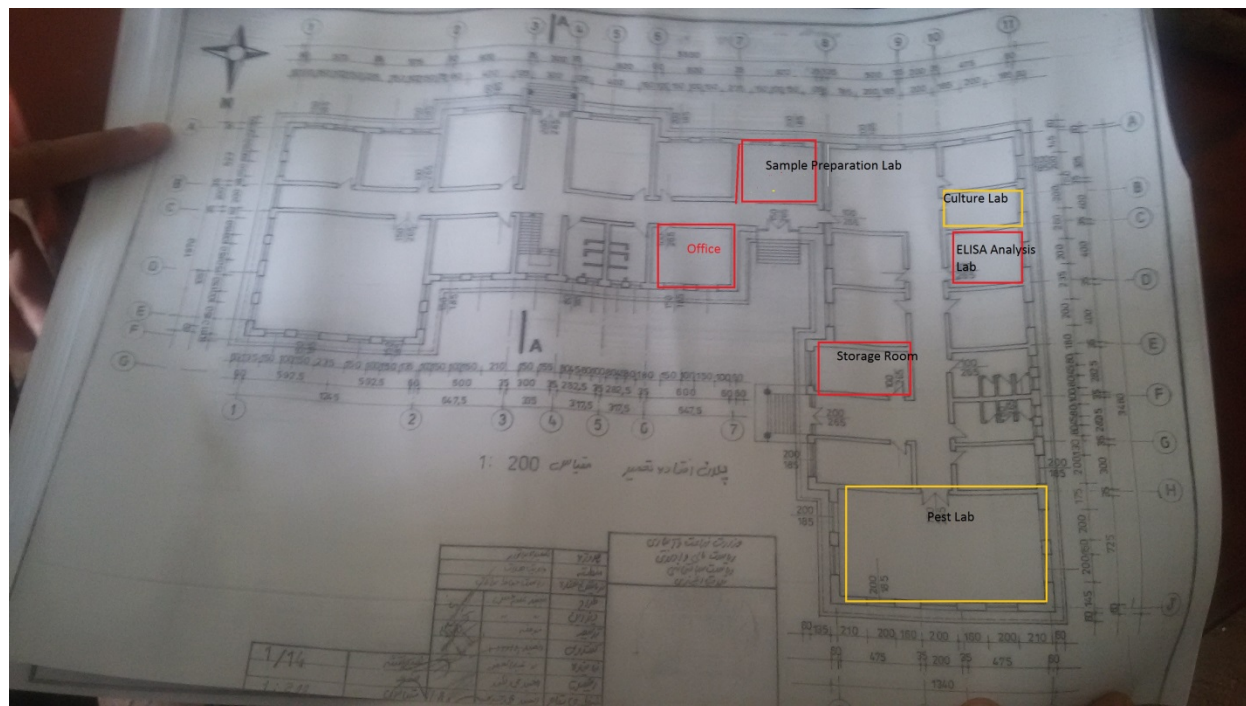


Figure 5: Blueprint of the PPQD laboratory in Kabul, showing specific areas occupied by the mycotoxin laboratory.

Challenges

Inconsistent power in Afghanistan is a constant problem when conducting sensitive tests. Hopefully the stabilizers and backup batteries provided by the project will provide adequate and stable power for execution of the analysis.

2. Implementation of Data Collection and Sample Analysis

2.1 Implementation of sample collection

Activities

The PHL Innovation Lab Afghanistan project team will work with MAIL-PPQD and DAIL staff to sample and assay commodities located in various parts of the country. MAIL-PPQD staff time and facilities will serve as the GIRoA's in-kind contribution to this assessment project.

The sampling plan for the entire project was developed based on consumer consumption patterns and export importance of specific commodities. The total number of samples collected for analysis will be 500 to 800; with 30% flour and wheat products, 23% raisins, 18% almonds, 18% pistachios, and 11% walnuts. GPS coordinates will be recorded at every site for follow-up.

The wheat sampling plan was determined based on the data gathered by USDA – Foreign Agricultural Service – Global Agricultural Information Network (USDA-FAS-GAIN) which indicates that 80% of all wheat and wheat food products is of Afghan origin, 10% is Pakistani flour, 4% is of Kazakhstan origin, 3% is of Uzbekistan origin, and 3% is of Iranian origin. A total of 75% of all flour milled in Afghanistan is milled in the small asiab mills (stone mills) or grist mills (small, portable, roller mills). The wheat samplings were divided according to reported consumption habits and production (Table 2). Wheat sampling will occur in Kabul, Kunduz, Herat, Kandahar, Parwan, and Mazar-i Sharif.

Table 2: Proposed Wheat / Flour / Wheat Products Sampling Scheme.

Type of Samples	Number of Samples
Asiab and Grist Mill Flour of Afghan Origin	104 - 144
Asiab and Grist Mill Flour of Kazakhstan Origin	5 - 9
Asiab and Grist Mill Flour of Uzbekistan Origin	3 - 6
Purdue Improved Crop Storage (PICS) bags	2 - 4
Two warehouses or storage facilities in Mazar-i-Sharif, Kabul, Kunduz, and Herat, as recommended by grain traders or farmers.	8
Two naan bakeries in each of the four regions	8 - 20
Two – four flour millers in each of the four regions	8 - 20
In Kabul market sampling of Pakistan flour	6 - 12
In Herat market sampling of Iran wheat products	3 - 6
In Kabul market sampling of other flour	3 - 6
TOTAL	150 - 235

Raisins have high potential for export into the international markets. The Afghan raisins have unique flavor and variety. Afghan raisins have a high degree of Brix (fructose levels). Raisin sampling will occur in Kabul, Shamali Plain, Herat, Mazar-I Sharif, and Kandahar. The seven major varieties of raisins will be sampled as illustrated in the Table 3.

Table 3: Proposed Raisins Sampling Scheme.

Type of Samples	Number of Samples
Medium Quality Round Green Raisin	18 - 27
Medium Quality Long Green Seedless Raisin	18 - 27
High Quality Shundurkhani Raisin (golden-high value)	16 - 26
Medium Quality Red Raisin	12 - 27
Sun dried Shomali Raisin (dark red)	15 - 27
Sun dried Ghazni Raisin (dark red)	15 - 27
Sun dried Tayefe Raisin (Mazar-i-Sharif - rose colored)	15 - 27
TOTAL	110 - 188

Almonds, pistachios and walnuts have high potential for export into the international markets. Afghan nuts have unique flavor and variety. Afghan almond flavonoids are more bioactive than almonds grown in the West. Almonds with these high levels of flavonoids could be niche marketed if these almonds could meet international phytosanitary standards. The major varieties of nuts will be sampled as illustrated in Tables 4-6.

Table 4: Proposed Almond Sampling Scheme.

Type of Samples	Number of Samples
Sattarbai Soft-shell Almonds (Mazar-i-Sharif)	15 - 24
Shokorbai Hard-shell Almonds	15 - 24
Abdul Wahidi Almonds (Mazar-i-Sharif)	15 - 24
Qambari Almonds	15 - 24
Ghorbandi Almonds	15 - 24
Sangaki and Murawaji Almonds (smaller kernels)	15 - 21
TOTAL	90 - 141

Table 5: Proposed Pistachio Sampling Scheme.

Type of Samples	Number of Samples
Korak Pistachios	23 - 38
Pushdara Pistachios	23 - 38
Khandan-e-safid Pistachios	23 - 38
Other varieties of Pistachios	21 - 36
TOTAL	90 - 142

Table 6: Proposed Walnut Sampling Scheme.

Type of Samples	Number of Samples
Zard Walnuts (yellow kernels)	10 - 17
Mazaari Walnuts (yellow kernels)	10 - 17
Takhari Walnuts	10 - 17
Korek Walnuts	10 - 17
Kaghazi Walnuts (paper shelled)	10 - 17
Other varieties of Walnuts	10 - 15
TOTAL	60 - 94

Sampling protocols have been developed for all commodities of interest, as previously mentioned in this progress report. As the samples arrive from the field in the Kabul MAIL laboratory, the assays will be conducted starting with the raisins because these samples would be the most susceptible ones to allow mold growth and toxin production during storage.

Progress

Sampling was conducted in Mazar-e-Sharif and in Balkh Province (Table 7) on August 2nd to the 5th, 2015. The UV lights to the right were used to identify both cross contamination and potential contamination of mycotoxins. The sampler discovered potential contamination on the walls and floor of the asiab flour mills. Also, there was potential contamination on cement walls and floors of the traditional raisin drying facilities. Most pistachios, walnuts, and almonds were from the previous year and are highly suspected to be contaminated with mycotoxins. The new harvest for pistachios, walnuts, and almonds has not begun. It is recommended by the sampling team to return to Mazar in September to gather new harvest pistachios, walnuts, and almonds and compare them to previous year's production.



Figure 6: Evaluation of potential *Aspergillus sp.* presence in samples.

Sampling was conducted in Herat and in Herat Province (Table 8) on August 9nd to the 13th, 2015. The sampler discovered potential contamination on the walls and floor of the asiab flour mills. Also, there was potential contamination on cement walls and floors of the traditional raisin drying facilities. Most pistachios, walnuts, and almonds were from the previous year and are highly suspected to be contaminated with mycotoxins. The new harvest for pistachios, walnuts, and almonds has not begun. It is recommended by the sampling team to return to Herat in September to gather new harvest pistachios, walnuts, and almonds and compare them to previous year's production



Figure 7: A typical grist mill in Herat Province

Table 7: Samples from Mazar-e-Sharif and Balkh Province.

Samples of dried fruits/nuts, wheat and wheat products collected in Mazar																		
Type of sample	Raisin		Almond						Pistachios				Walnuts					
	Sun dried Tayefe Raisin (rose colored)	Other Raisin	Sattarbai (soft-shell) Almonds	Shokorbai Hard-shell Almonds (5-8) A02	Abdul Wahidi (hard-shell) Almonds	Qambari (hard shell & exported) Almonds	Kherudini	Qaharbai	Sangaki and Murawaji Almonds (smaller kernels)	Korak (closed mouth) Pistachios	Pushdara (with skins or Shuli) Pistachios	Khandan-e-safid (open mouth) Pistachios	Other varieties of Pistachios	Mazaari Walnuts	Takhari Walnuts (white kernel & soft shell)	Kaghazi Walnuts (paper shelled)	Other varieties of Walnuts	
Sample Code	Ro7	R08	A01	A02	A03	A04	107	A08	A06	P01	P02	P03		WN02	WN03	WN05	WN06	
No. of Samples to be Collected	14-17	--	4-6	5-8	4-5	4-5	--	--	4-6	6-9	6-9	6-9	5-9	5-8	5-8	5-8	5-8	
Total Sample Collected			14	5	2	3	5	4	1	4	2	5	3	2	1	1	1	3
Total Sample	14		25						12				6					
Wheat and Wheat products																		
Type of Sample	Asib Mill	Grist Mill	Asiabs and Grist Mill Flour of Kazakhstan Origin (5) W03	Asiabs and Grist Mill Flour of Uzbekistan Origin (3) W04	Purdue Improved Crop Storage (PICS) bags	Warehouses or storage facilities	Naan bakeries	Commercial Flour Mill										
Sample Code	W01	W02	W03	W04	W05	W06	W07	W08										
No. of Samples to be Collected	(13-18)	(13-18)	5	3	2	2	2-5	2-5										
Total Wheat and Wheat Products Samples	35		1	1	0	2	3	6										

Table 8: Samples collected from Herat Province.

Samples of dried fruits/nuts, wheat and wheat products collected in Herat												
	Raisin			Almond				Pistachios			Walnuts	
Type of sample	Medium Quality round green raisin	Medium Quality long green seedless raisin	Other raisin	Abdul Wahidi (hard-shell) Almonds	Sangaki and Murawaji Almonds (smaller kernels)	Other Almond	Sangaki and Murawaji Almonds (smaller kernels)	Korak (closed mouth) Pistachios	Pushdara (with skins or Shuli) Pistachios	Khandan-e-safid (open mouth) Pistachios	Korak Walnuts	Other varieties of Walnuts
Sample code	R01	R02		A03	A06		A06	P01	P02	P03	WN05	WN06
No. of samples to be collected	5-7	5-7		4-5	2-6		4-6	6-9	5-9	5-9	5-8	3-5
Total sample collected	7	10	4	4	5	2	4	4	1	4	2	4
Total sample	21			11				9			6	

Wheat and Wheat products													
Type of sample and	Asiab Mill	Grist Mill	Asiabs and Grist Mill Flour of Kazakhstan Origin (5) W03	Asiabs and Grist Mill Flour of Turkmanistan Origin (3)	Asiabs and Grist Mill Flour of Pakistan	Purdue Improved Crop Storage (PICS) bags	Commercial flour mill						
Sample code	W01	W02	W03	W07	W09	W05	W08						
No. of samples to be collected	(13-18)	(13-18)	5	3	2	2	2--5						
Total sample collected	8	11	4	3	2	2	6						
Total wheat and wheat products samples	8	11	4	3	2	2	6						

Challenges

Security remains a challenge when sampling. PHL-IL reviews all security reports for the given area and constructs the sampling plan accordingly. Unfortunately, PHL-IL has not been able to access some of the remote areas because of security concerns.

2.2. Implement sample analysis and data collection

Activities

Based on the literature review, the climate prevalent in the country and available epidemiological data related to cancer in Afghan's population, the mycotoxins of concern in the commodities of interest were defined and the testing methods chosen.

The following mycotoxin test kits from Romer Labs were chosen for this project:

- AgraStrip for DON to be used on wheat analysis
- AgraQuant for AFL to be used on wheat analysis
- AgraQuant for OTA to be used on wheat analysis
- AgraQuant for T2 to be used on wheat analysis
- AgraQtrip for AFL to be used on the analysis of raisins and nuts
- AgraQuant for OTA to be used on the analysis of raisins and nuts

The above Romer kits have met or exceeded the International Standard ISO/IEC 17025:2005 and ISO 9001:2008 Quality Management Systems — Requirements.

All chosen kits have been validated by the manufacturer (Romer Labs) for the use on the evaluation of mycotoxin levels in the commodities of interest to this project. Protocols related to the use of the test kits chosen for the project were prepared to standardize the quantification of mycotoxins in the samples.

Challenges

Significant sample analysis and data collects as of August 31, 2015 occurred because of purchase and installation of power equipment. The original timeline had full analysis being conducted by the 30th of July, 2015 but analysis was not fully functional until August 30, 2015. The MAIL-PPQD staff has limited laboratory experience and error and reruns have been frequent during preliminary analysis.

3. *Generate Project Reports and Disseminate Findings*

3.1 Generate progress and final report which will summarize findings and propose recommendations for the follow-up actions

Activities

The PHL Innovation Lab Afghanistan project team will generate progress and final reports that will summarize findings and propose recommendations for follow up actions. The final report is to be shared with MAIL, MoCI, MoPH, and WFP.

Progress

This progress reports is the first one submitted.

Challenges

N/A

3.2 *Support the organization of an international workshop on the reduction of post-harvest losses outside of Afghanistan.*

Activities

The PHL Innovation Lab Afghanistan project team will provide administrative and technical support for organizing an international workshop in support of addressing pre- and post-harvest losses with a special emphasis on Afghanistan. This workshop will take place outside of Afghanistan near the end of this project.

Progress

Nothing to report at this time.

Challenges

N/A

B. Issues or concerns encountered during the reporting period

- Constant attendance by MAIL PPQD staff for work in the laboratory. Those that show up for work receive incentive pay and those that do not show up for work do not receive incentive pay.
- Various levels of commitment at MAIL PPQD.
- Sample preparation is considered tedious at times.
- Protocols have been finalized and have been submitted to MAIL, translated, and utilized in staff training.
- Recent discussions during weekly project conference calls revealed that disbursement of travel funds including per diem for MAIL staff efficiently continues to be an issue while in Afghanistan.
- As a result of project delays, a revised timeline with a proposed no cost extension for the current project through February 28, 2016 is included with this progress report.
- As a result of project delays, additional effort by project collaborators and changes in anticipated project personnel and partners, a revised budget for the current project through February 28, 2016 is included with this progress report. The subcontract amount to University of Nebraska Lincoln has been substantially increased because the project Co-lead PI (Bianchini) and her staff have spent considerably more time and effort on project planning, supplies and materials identification and order preparation, and training of the project coordinator than initially planned. The total budget allocated for this project remains unchanged.

C. Data Sharing and Dissemination

No progress to report in this area.

II. Human and Institutional Capacity Development

A. Short-term training

- See activity 1.2 and 1.3 in terms of training MAIL staff to collect samples along the value chains, to receive samples and prepare them for analysis, and to analyze samples for mycotoxin presence with the provided equipment and according to standard laboratory practices.

B. Long-term training

- There are no plans in the current project for long-term training.

C. Institutional capacity development

- See activity 1.4 in terms of equipping a designated mycotoxin laboratory in MAIL for the purpose of providing food safety analysis services to the value chains beyond the life of this current project.

III. Technology Transfer and Scaling Partnerships

- The primary technology transfer will be to MAIL in terms of equipping a mycotoxin testing laboratory and training technical staff to operate this lab according to internationally accepted standards.

IV. Future Work

- Depending on the results of this value chain assessment a follow-up project may be developed and recommended.



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FROM THE AMERICAN PEOPLE

FACT SHEET

Date: March 2016

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Website: <http://www.usaid.gov/afghanistan>

Project Name: PHL Innovation Lab – Afghanistan Mycotoxin Value Chain Project

Implementation period: 1 Jan 2015 – 30 April 2016

Project budget: \$1,220,535

OVERVIEW

Mycotoxins are noxious chemicals synthesized by some fungi when grains and other foods are stored improperly. At their worst mycotoxins are lethal for humans and domesticated animals. More commonly they are debilitating and are associated with reduced functioning of critical organs, e.g., liver, kidneys, immune system, etc., increased cancer risks, and stunting in children under age five. Mycotoxins are regulated in international trade and may be associated with major economic losses if foods are deemed contaminated and must either be destroyed, returned to the location of export, or devalued for uses other than originally intended. Mycotoxin contamination has hindered export of raisins and tree nuts from Afghanistan. The large amounts of wheat consumed daily by most Afghans increases potential exposure to some toxins far beyond that which occurs in most developed countries. Stress from drought, excessive heat and insects can increase mycotoxin contamination.

The USAID-sponsored Feed the Future Reduction of Post Harvest Losses Innovation Lab (PHL) will provide technical expertise to design and implement a mycotoxin assessment in collaboration with the Afghanistan Ministry of Agriculture, Irrigation and Livestock (MAIL). The assessment will include almonds, pistachios, walnuts, raisins and wheat in Afghanistan. As part of the assessment, a lab will be established and staff from MAIL trained to conduct entry level mycotoxin screening protocols. Results obtained will be used to increase food safety and security within the country, and to reduce problems encountered by exporters selling Afghan nuts and raisins in international markets.

ACTIVITIES

- Established and equipped an entry level mycotoxin screening lab in collaboration with MAIL in Kabul.
- Trained MAIL staff to collect field samples and lab technicians to run assays with commercially available test kits
- Surveyed wheat, raisins and tree nuts for mycotoxins commonly recovered in temperate climates.
- Verified accuracy of Afghanistan analyses in leading laboratories in Europe and the United States.
- Analyze samples collected for less common mycotoxins to ensure the mycotoxins of greatest importance in Afghanistan have been identified.
- Host a policy and technical workshop to disseminate study findings and to chart the road forward.
- Develop a mycotoxin mitigation plan and focused research to determine the origins of and solutions for the identified mycotoxin problems in Afghanistan

RESULTS

- Equipped a MAIL lab for conducting routine commercial mycotoxin assays as first line screens for contamination.
- Trained 42 MAIL staff to collect samples for mycotoxin assessments and 14 lab technicians to run commercially available tests to identify commonly occurring mycotoxins.
- Conducted a half-day workshop at the Faculty of Agriculture of Kabul University (approximately 500 participants).
- Debriefed MAIL and USAID on the study progress and accomplishments
- Determined that commercial test kits for T-2 and HT-2 are inadequate for analysis of wheat samples from Afghanistan
- **Almonds** – 15/81 samples contaminated with aflatoxins at an export limiting level; no detectable ochratoxin.
- **Pistachios** – 19/40 samples contaminated with aflatoxins at an export limiting level; 2/40 samples had significant ochratoxin contamination.
- **Raisins** – 43/89 samples contaminated with aflatoxins at an export limiting level; 25/80 samples contaminated with ochratoxin at an export limiting level. Results from Austria and Afghanistan are discordant.
- **Walnuts** – 8/25 samples contaminated with aflatoxins at an export limiting level; no samples with ochratoxin contamination at an export limiting value.
- **Wheat** – 23/151 samples marginally contaminated with aflatoxins; 3/185 samples contaminated with deoxynivalenol (DON) above international safety limits; 36/181 samples marginally contaminated with ochratoxin A. Identified ergot alkaloids in 51/151 samples and as a major unexpected health risk for many residents of Afghanistan.

Appendix VII –

Excel spread sheet with results from the multi-mycotoxin analysis

VII.1 Sheet 1 – Wheat

VII.2 Sheet 2 – Nuts

VII.3 Sheet 3 – Raisins

**Appendix VIII –
Excel spread sheet with complete data set for screened toxins**

VIII.1 Sheet 1 – Almonds

VIII.2 Sheet 2 – Pistachios

VIII.3 Sheet 3 – Raisins

VIII.4 Sheet 4 – Wheat and flour

VIII.5 Sheet 5 – Walnuts

**Appendix IX –
Metabolites detected with fungal genus that commonly produces the**

Commodity	Fusarium metabolites	Alternaria metabolites	Aspergillus metabolites	Penicillium metabolites
Wheat	Beauvericin	Tenuazonic acid	3-Nitropropionic acid	Mycophenolic acid
	Enniatin A	Alternariol	Kojic acid	Agroclavine
	Enniatin A1	Alternariolmethylether	Sterigmatocystin	Chanoclavin
	Enniatin B	Tentoxin	Methoxysterigmatocystin	Elymoclavine
	Enniatin B1	Altersetin	Averantin	Citrinin
	Epiequisetin	Altersolanol	Averufin	Secalonic acid D
	Equisetin	Altertoxin-I	Norsolorinic acid	Questiomycin A
	Chrysogin	Macrosporin	Cycloaspeptide A	Quinolactacin A
Nuts	Zearalenone-sulfate	Tenuazonic acid	Cyclopiazonsäure	Mycophenolic acid
	alpha-Zearalenol	Alternariol	Kojic acid	Mycophenolic acid IV
	beta-Zearalenol	Alternariolmethylether	3-Nitropropionic acid	Penitrem A
	HT-2 toxin	Altersetin	Asperfuran	Agroclavine
	T-2 toxin	Tentoxin	Paspalin	Chanoclavin
	Butenolid	Macrosporin	Nigragillin	Festuclavine
	Epiequisetin	Infectopyron	Malformin A	Epoxyagroclavin
	Equisetin		Malformin A2	Andrastin A
	Fusaric acid		Malformin C	Andrastin B
Grapes		Tenuazonic acid	Malformin A	Mycophenolic acid
		Alternariol	Malformin A2	Mycophenolic acid IV
		Alternariolmethylether	Malformin C	Quinolactacin A
		Altersetin	Pyranonigrin	Andrastin A
		Altertoxin-I	Nigragillin	Andrastin B
		Tentoxin	Aurasperon B	Andrastin C
		Macrosporin	Aurasperon C	Chanoclavin
			Aurasperon G	Festuclavine
		Fonsecin	Penitrem A	

Appendix X – Project time line – June 2015

Revised Time Line
for the
Rapid assessment of Mycotoxins in Afghanistan’s food value chains

I. TIMELINE ADJUSTMENT JUSTIFICATIONS

The main reasons for the “Timeline Adjustments” are:

- The Ochratoxin (OTA) enzyme-linked immunosorbent assay (ELISA) kits were in back order. The OTA ELISA Kits were manufactured in Singapore and arrived in the US the first part of May. This is the major mycotoxin of concern because of the linkage with liver and kidney cancer.
- The StatFax Readers, balances, and incubators for the AgraVision Readers all required 220 voltages, which were imported from the EU. These arrived mid-May.
- The Seedburo Hand-Held Ultraviolet Lamp was also on back order. The UV lamp is used to determine environmental contamination. These arrived mid-May.
- The water distillation system will not be delivered to the shipment container before June 19, 2015.
- The shipment is leaving for Kabul late June 2015 and will not arrive in Kabul until early July 2015.
- The clearance at Kabul Airport customs usually takes two weeks. The kits and the laboratory equipment will arrive at MAIL PPQD about the 19th of July 2015.
- July 10th, 2015 is the last week of Ramadan and EID concludes on the 21st of July, 2015. Afghans fasts from sunrise to sunset, which means the Afghans do not eat or drink anything, including water. The Afghans only sleep 3 hours a night, which is ineffective to start a detailed project.
- Recommended start date for training and sampling by MAIL staff is July 25th, 2015.
- The Project Manager’s VISA was only approved the week of May 25th.
- The 2-month shift of the project timeline moves the preparation and organization of the international workshop into the latter part of the fall semester (when at least one key project team member [Bianchini] has a conflict with UNL on-campus teaching duties] and the U.S. holiday season. Thus, holding of the international workshop is proposed in early to mid-January.
- Project conclusion is therefore proposed to be shifted from December 31, 2015 to February 28, 2016.

Considering these time constraints the PHL Innovation Lab Afghanistan project team requests approval of the proposed revised schedule.

II. ILLUSTRATIVE DURATION, TIMING AND SCHEDULE

Task	LOE	Estimated Schedule
Pre-project scope of work development and initial research methodology development	4 weeks	January 1-30, 2015
Desk study. Review available reports related to assignment	1 week	February 2-5, 2015
Consultation with MAIL, USAID other stakeholders via conference calls	weekly	Since February 9, 2015
Development of research methodology	3 weeks	February 16-March 6, 2015
Confirmation of approach and preparation to undertake assessment	8 weeks	March 9-May 1, 2015
Procurement of assay kits and lab equipment	4 weeks	May 4-June 19, 2015
Shipment of assay kits and lab equipment to Afghanistan	3 weeks	June 20-July 5, 2015
Kabul Airport Customs Clearance	2 weeks	July 5-July 19, 2015
End of Ramadan		July 16, 2015
Eid al-Fitr	5 days	July 17-21, 2015
Departure for Afghanistan	2 days	July 17-19, 2015
Set up Mycotoxin Lab at Badam Baugh	5 days	July 20-23, 2015
Training of assayists and sample collectors	1 week	July 25-29, 2015
Sample collection Mazar i-Sharif, Herat, Kunduz, Kandahar, Shamali Plain, and Kabul	6 weeks	August 1-September 9, 2015
Detail training on Sample Analysis and Assays	1 week	August 1-5, 2015
Training of assayists and sample collectors (Kunduz & Kandahar)	1 week	August 15-19, 2015
Lab Sample Analysis	6 weeks	August 8-September 30, 2015
Preliminary Analysis of data	2 weeks	September 19-October 6, 2015
Eid al-Adha	3 days	September 22-23, 2015
Pre-departure briefing	1 day	October 5, 2015
Lab Equipment Inventory	1 week	October 1-6, 2015
Departure from Afghanistan, delivery of select samples to Austrian Lab, and return to USA	4 days	October 7-10, 2015
Finalization and submission of mycotoxin assessment report and recommendations	6 weeks	October 12-November 28, 2015
Organizing international workshop including support for obtaining travel permissions for Afghan MAIL staff to Abu Dhabi	4 weeks	December 2, 2015-January 3, 2016
International Workshop (Abu Dhabi)	2 days	January 4-6 or 11-13, 2016
Finalization of overall project report and final accounting	6 weeks	February 28, 2016

Appendix XI – Departure Debriefing for USAID – December 2015



بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

In the name of Allah

Presentation on mycotoxin

Prepared by

Asadullah Ansari

Zakia Stanekzy

Jahid Ahadi









Mycotoxins: An Overview

Andreia Bianchini, PhD

University of Nebraska – Lincoln

and

Debra Frey, MSc

Kansas State University



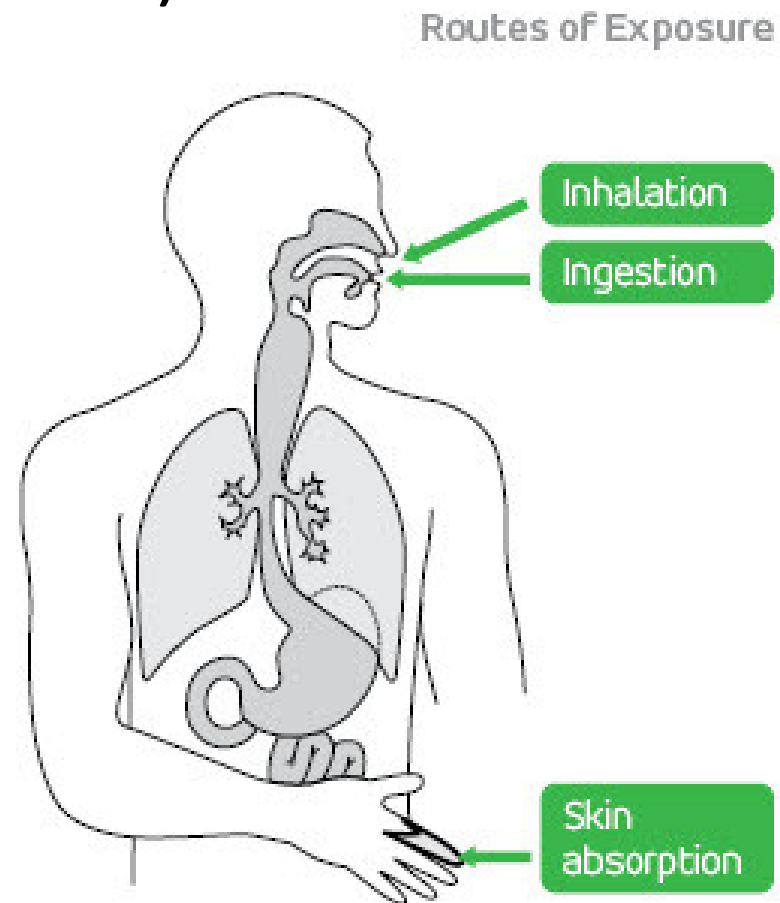
General Information

- Large, diverse group of fungal toxins
- Naturally occurring
- Toxic to plants, animals, humans, microorganisms and cell cultures
- May be thousands of unique mycotoxins in nature
- True number is unknown



Exposure

- Ingestion (Direct or Indirect)
- Inhalation
- Direct dermal contact



Concerns About Mycotoxins

- Where populations have a single dietary staple

- May be exposed to great amounts
- Acute and chronic toxicity possible
- Less developed countries – more direct exposure



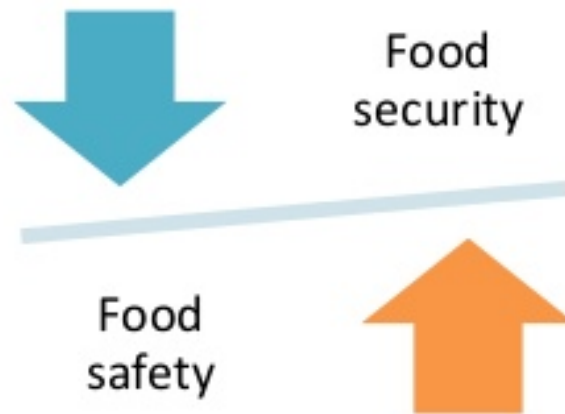
- Where diets are diverse

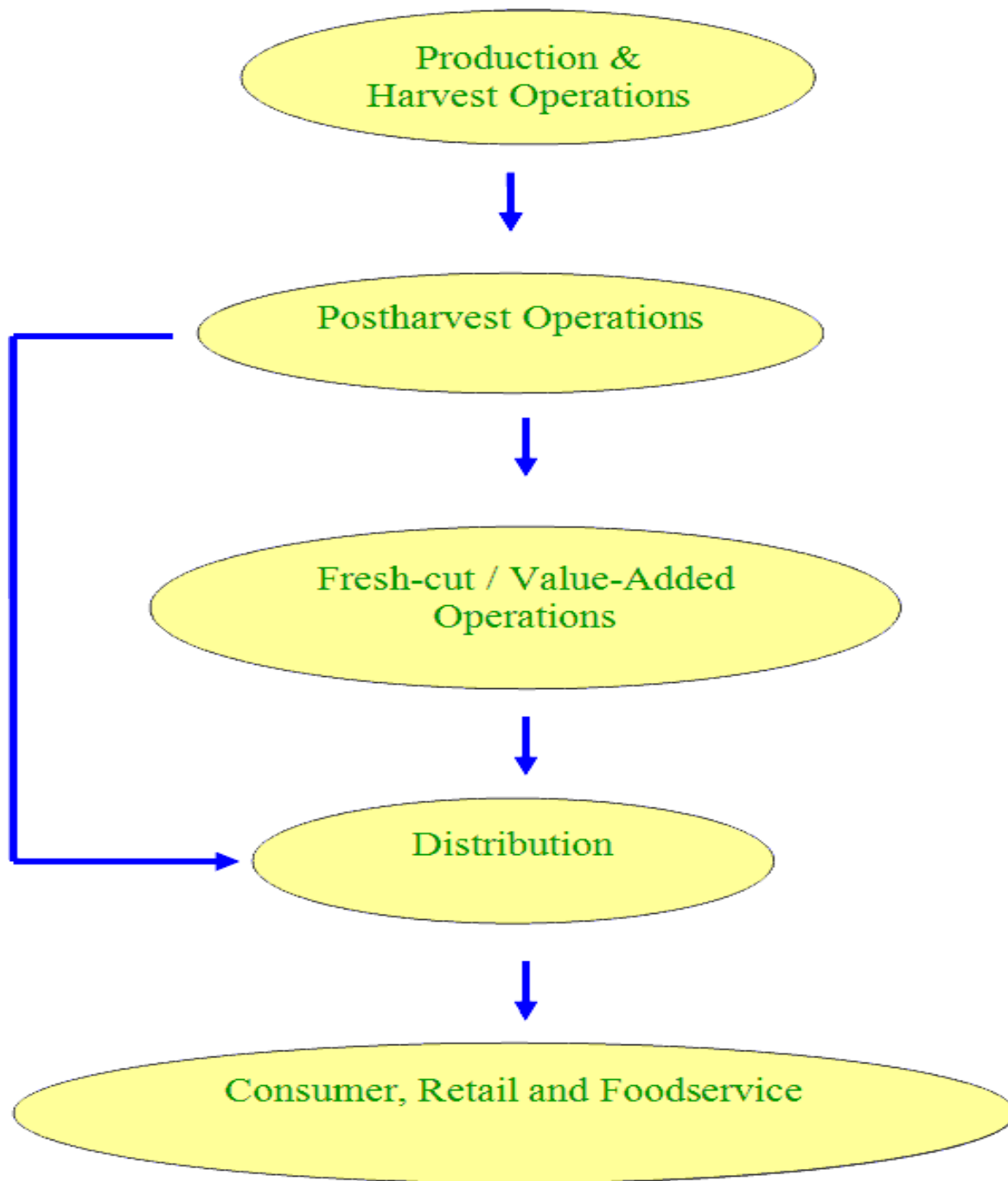
- Low levels of exposure
- Foods of better quality – lower amounts
- More developed countries – direct and indirect exposure



- ❖ Food Ingredients
- ❖ Residues in animal products – milk, eggs, edible organ tissues

Mycotoxins: a multi-disciplinary issue





Sampling

سَمپل گَیری

Presented by:

Asadullah Ansari

MSc Plant Pathology

Preface مقدمه

Afghanistan depends on agricultural products.

- Main source of national income.
- 85% people directly and indirectly involve in agriculture.
- Plant production is the back bone of Afghan export

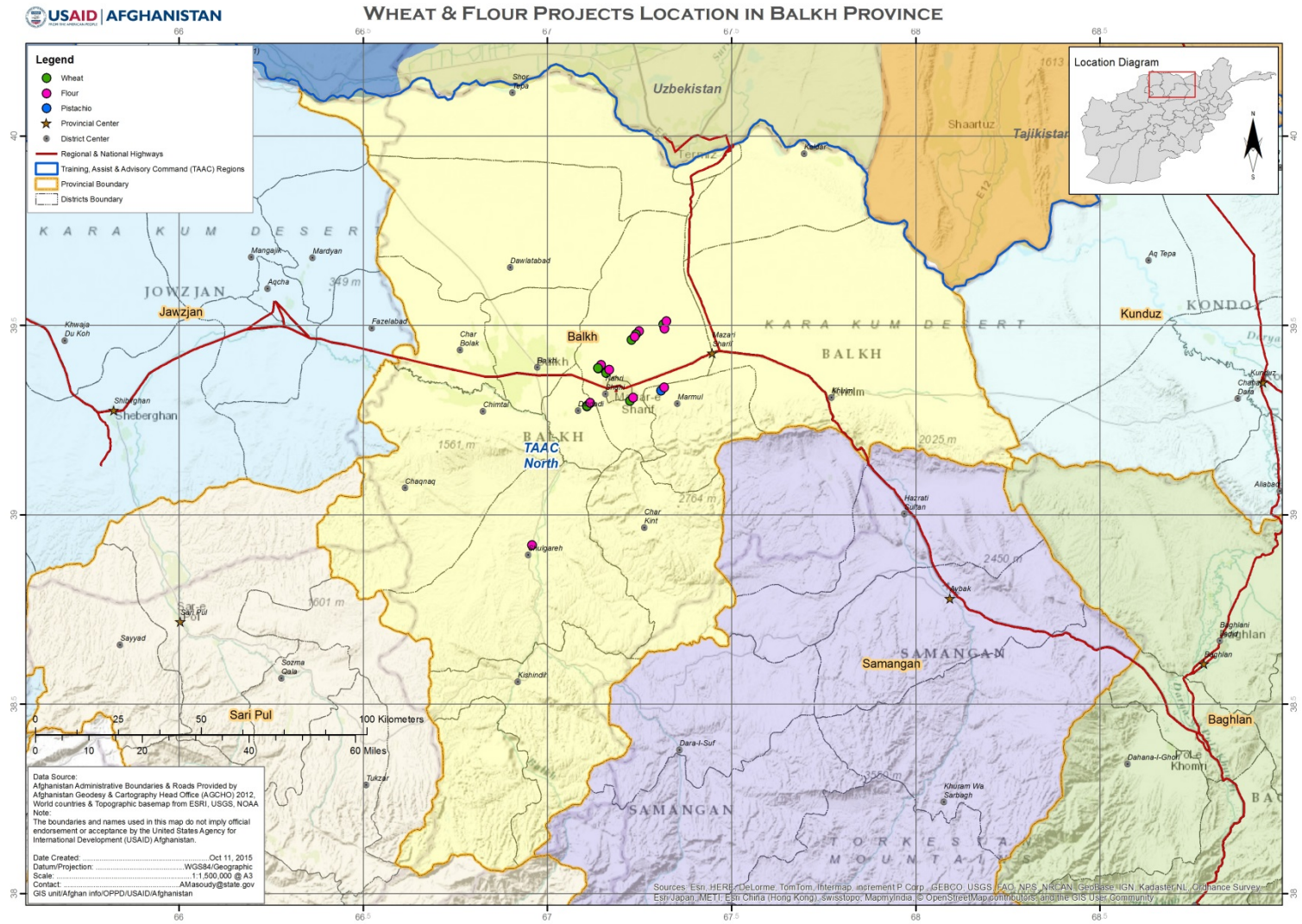
Sampling is a method of studying from a few selected items, representing the entire number of units or lot.



Provinces

Samples were Collected

Herat, Kandahar, Balkh, Samangan, Kabul, Kapisa, and Parwan.



Standard method for sample collection

- ▶ Pistachios, Almond and Walnuts Kernel. 1-kg
- ▶ Pistachios, Almond and Walnuts with shell 2-kg
- ▶ Raisins 1-Kg
- ▶ Wheat Flour 1- Kg.



هدف

Objective

Sample collection for research on mycotoxins.

- Storage observation .
- Local standards.
- Storage duration.
- Storage sanitation.

Pre-Sampling preparation

1. Selection area.
2. Equipments collection bags, gloves,
3. Mask, transferring bag, UV-light, sample ID form, pen, camera, wet tissue paper, beaker, sample stick, GPS.



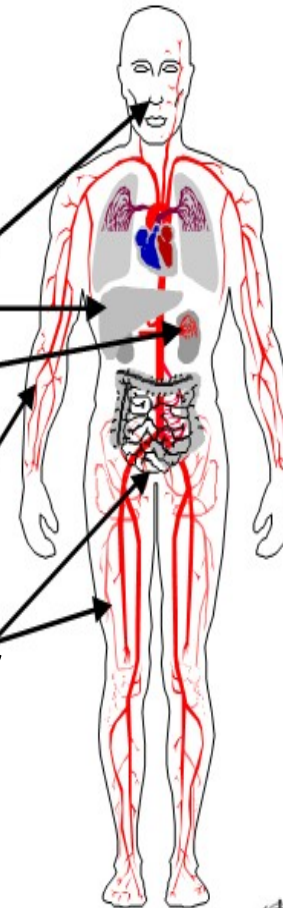
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Target organs of some mycotoxins

<i>Mycotoxin</i>	<i>Target</i>
Aflatoxin	liver
Ochratoxin A	kidney
Trichothecenes	mucosa
Ergot alkaloids	peripheral vascular system
Zearalenone	uro-genital tract





از توجه تان تشکر

THANKS

مايكوتاكسين (Mycotoxin)

مايكوتاكسين (Mycotoxin):

Aflatoxin (*A. parasiticus* *Aspergillus flavus*)

Ochratoxin (*Aspergillus ochraceus*, *Aspergillus carbonarius*).

(T2) *Fusarium* sp

DON(*F. graminearum* and *F. culmorum*)



Mycotoxin, Analyses procedure.

1- 300 g of sample

2- 450 Distilled water for raisins and nuts

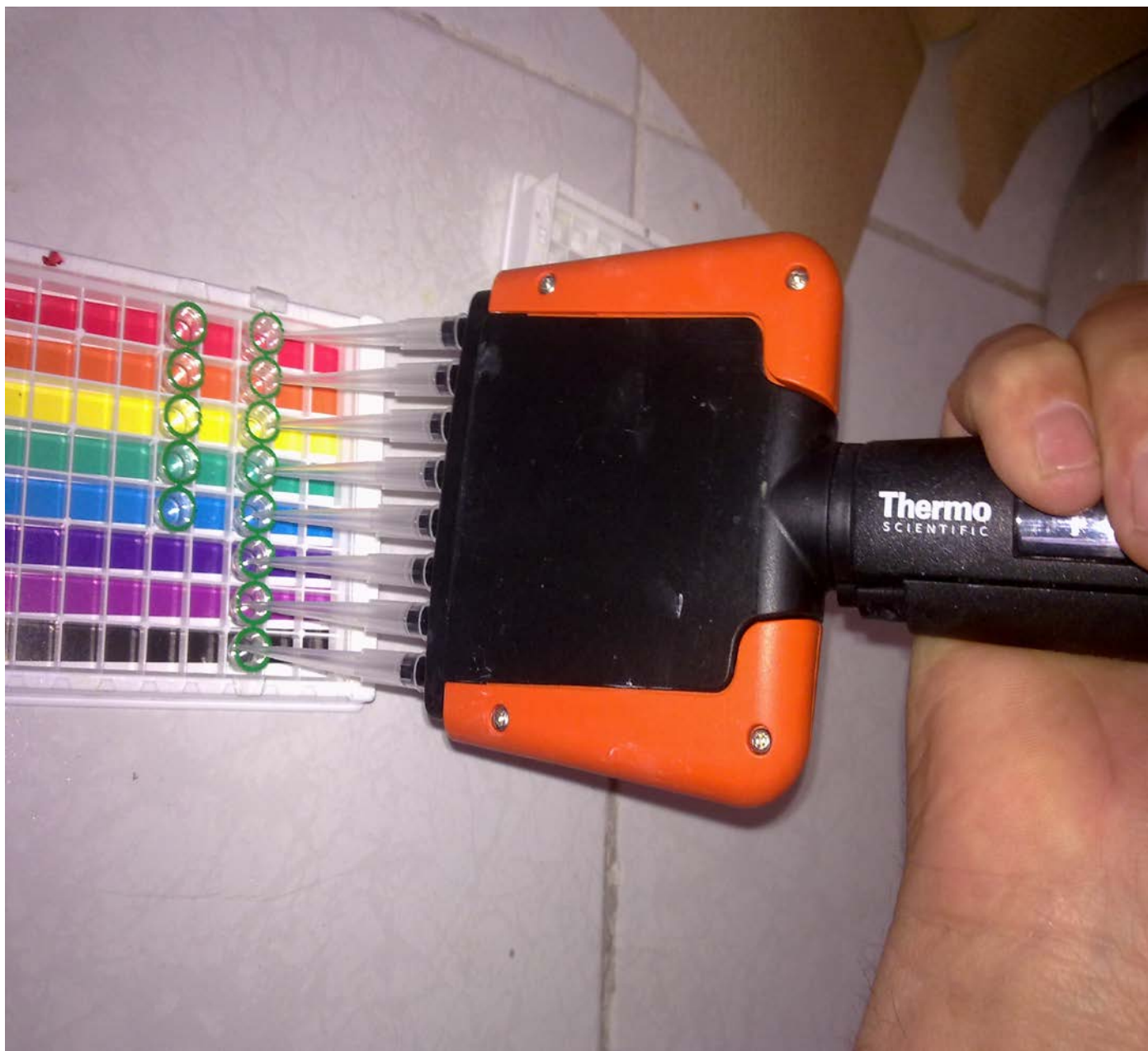
3- MIX, 5 minutes, 20g Extract + 100 μ l Methanol

70%. 3minutes, MIX - **Filtration**

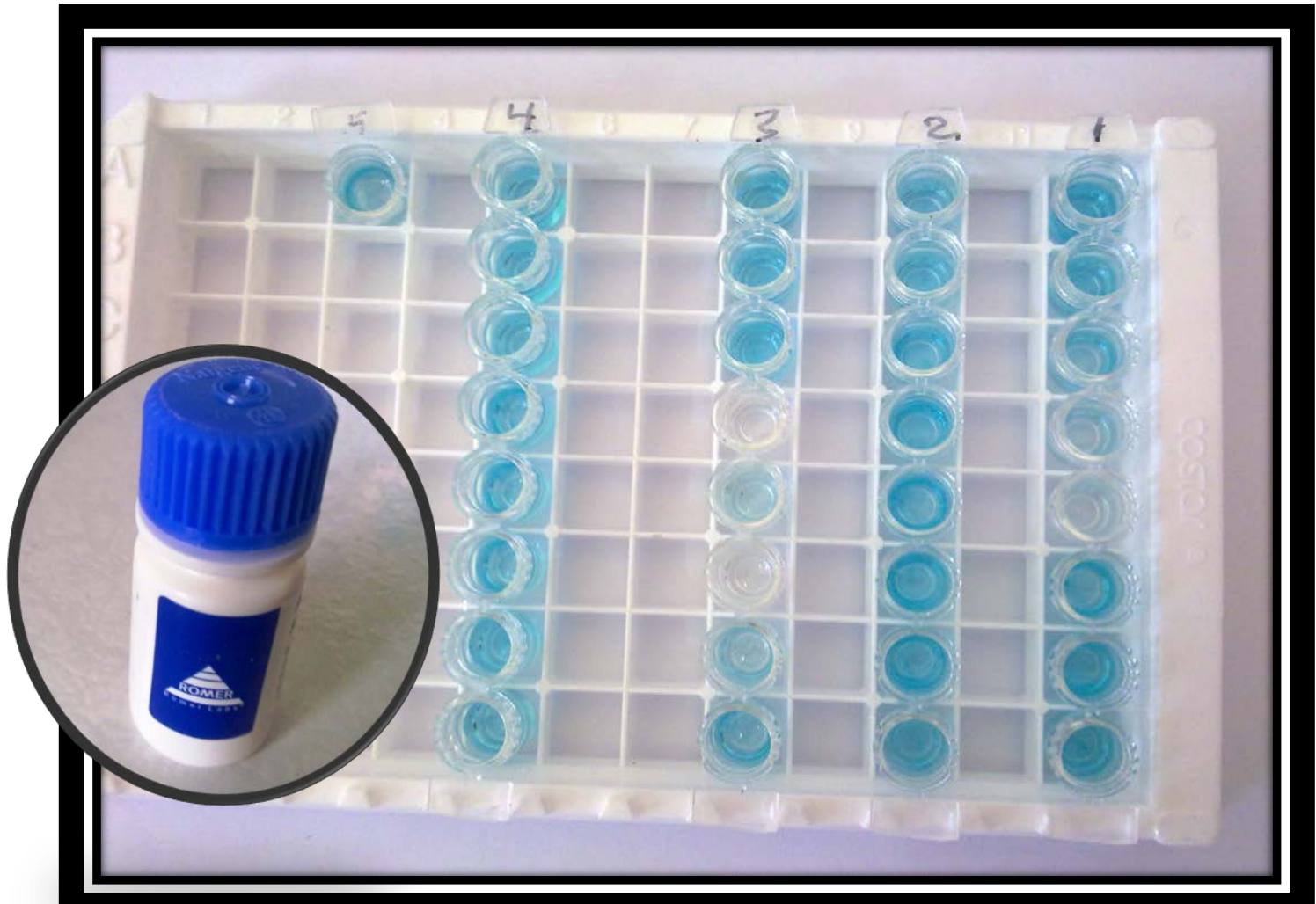


4- Elute pH is measured, ready for analysis, 4000 μ l Elute, Filtration, 200 μ l conjugate, 100 μ l standards, and 100 μ l Elute. MIX, Transfer 100 μ l, Antibody well. 15M, 5times wash, 100 μ l substrate 5 minutes incubation, 100 μ l stop solution .

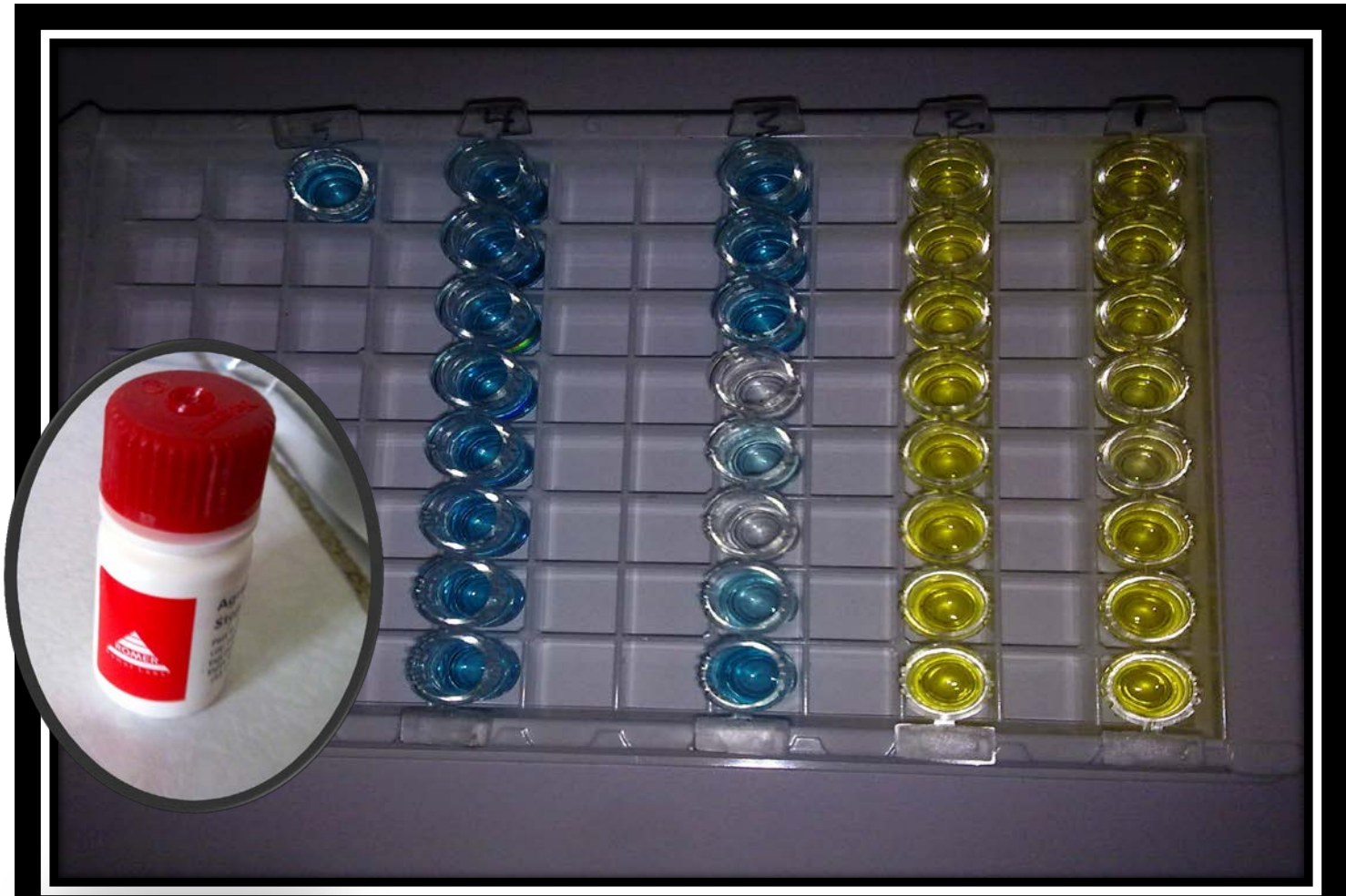




- Pipette 100 μL of the Substrate into each microwell strip using an 8-channel pipette. Incubate at room temperature for 5 minutes.



- Pipette 100 μ L of Stop Solution into each microwell strip using an 8-channel pipettor. The color should change from blue to yellow.





By
Zakia Stanikzai

Mycotoxin, impact on plant products export and import

Aflatoxin, ochratoxin

**Raisins, Nuts pistachios, Almond, walnuts
and etc.**

Max. permissible level

by food law and food safety by European Food
Safety Authority

8.0-10.0 ppb, $\mu\text{g}/\text{kg}$

Max. permissible level

by FDA: 20.0 ppb, $\mu\text{g}/\text{kg}$

Raisins and

Afghanistan exporting Raisins Nut's to 50 countries

2014 (CSO)			
NO	Plant Name	Quantity / Ton	Cost , US \$
1	Red Raisins	1012871	19,244,563
2	Green Raisins	758	1,667,705
3	Black Raisins	3716	5,203,751
4	Golden Raisins (Abjosh)	4525	11,260,397
Total		1021870	37,376,416

2014 (CSO)			
NO	Plant Name	Quantity / Ton	Cost , US \$
1	Shell Almond	1924	4,602,196
2	Almond Kernel	1661	1,2617,000
3	Pistachios Kernel	16311	22,517,000
4	Walnut Kernel	2648	9,048,107
Total		20620	44,182,107

Record of Rejection :

Raisins and pistachios several times.

Analytical results, over 18.0 ppb .

Transportation cost for one containers 20ft.

US \$10,000

Organization or company analyzing the mycotoxin

1- Afghan Raisin Fruits & Vegetables promotion administration

2- Company (Sun power).

Beneficiary export

- Reliability AF plant products (Safety).
- Improve the skill of PPQD staffs
- Access to more international Market (increase farmers' income) .
- Safety of public

Project objective

Assessment (Done) ارزیابی تشخیص

Mitigation : ؟ کاهش

Verification : ؟ تأیید

The end

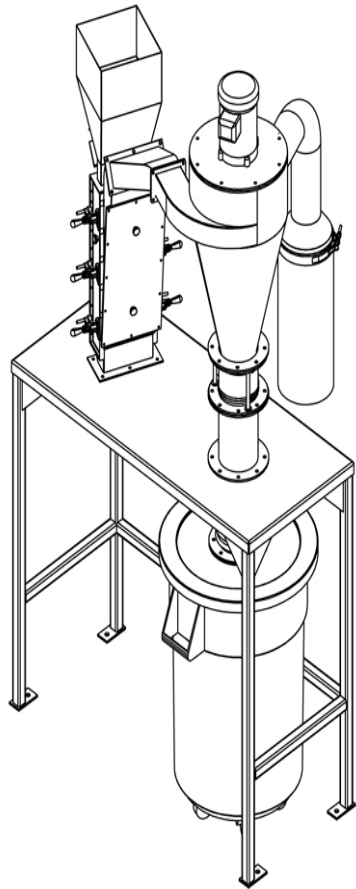
HOPE TO HAVE SAFE FOOD FOR EVERY ONE

Jahed AHADI

ماشین جداکن دانه های گندم مصاب

Air Aspirator for nuts & grains

<http://www.kice.com/Product-MultiAspirators.html>



Codex Discussion

- Review of Codex
- CODEX GENERAL STANDARD FOR CONTAMINANTS AND TOXINS IN FOOD AND FEED - CODEX STAN 193-1995
- CODEX STANDARD FOR WHEAT FLOUR - CODEX STAN 152-1985

Appendix XII – Delhi meeting report and presentations

- XII.1 Final meeting report
- XII.2 Agenda
- XII.3 Presentation 01-04 – Floros
- XII.4 Presentation 03-01 – Homer
- XII.5 Presentation 03-02 – Alamazi
- XII.6 Presentation 03-03 – Ahadi
- XII.7 Presentation 03-04 – Leslie
- XII.8 Presentation T-01 – Ansari
- XII.9 Presentation T-02 – Rustami
- XII.10 Presentation T-03 – Bianchini & Sabillon
- XII.11 Presentation 04-01 – Kablan
- XII.12 Presentation 04-02 – Logrieco
- II.13 Presentation 07-01 – Zedek
- XII.14 Presentation 07-02 – Johnson
- XII.15 Presentation 07-03 – Bandyopadhyay
- XII.16 Presentation 07-04 – Floros
- XII.17 Presentation 10-01 – Leslie & Floros
- XII.18 Presentation 10-02 – Leslie
- XII.19 Nominal Group Technique Discussion Guidelines
- XII.20 Nominal Group Technique Questions and Responses

Afghanistan Food Safety Meeting – 14-16 March 2016

Purpose and Design of the meeting

The meeting consisted of three primary activities:

- Presentations on topics of relevance to the mycotoxin project and future activities to be undertaken in Afghanistan by project participants and by selected individuals from outside this group. An agenda for the meeting and pdf files containing all of the presentations are attached.
- Field trips to visit the Indian Food Safety & Standards Authority, a commercial food testing laboratory, and the Airport Cargo Section at Ghandi International airport.
- Nominal Group Discussions. The Nominal Group discussion process was used to solicit responses to a number of critical questions regarding the current project and where further work could go in the future. A listing of the nominal group questions and the ranked/weighted responses to these questions are attached.

The meeting was attended by representatives from three ministries of the Government of Afghanistan – Ministry of Agriculture, Irrigation and Livestock, including Deputy Minister Haidari, the Ministry of Public Health, including Minister Feroz, and the Ministry of Commerce and Trade, as well as representatives from US universities, USAID offices in Washington, India and Afghanistan, private sector traders and testing laboratories, FAO, World Bank and additional NGOs. Meeting logistics were provided by USAID offices and staff based in Kabul and Delhi.

Presentations

Session 3 – Summary of Mycotoxin Assessment

McDonald Homer, from the Office of Agriculture at the USAID Mission in Kabul, made the first presentation. He noted that the rationale underlying the project was that exports of horticultural products from Afghanistan to the European Union were being rejected at a relatively high frequency due to excessive contamination with aflatoxin and ochratoxin. Questions also were raised about the potential role of mycotoxins in major health issues in the country such as stunting of children under age 5 (highest in the world), and the relatively high ranking of Afghanistan for liver disease (17th in the world) and kidney disease (16th in the world). Dr. Homer also explained how arrangements were made through USAID's Bureau of Food Security to fund an assessment of mycotoxin contamination of raisins, tree nuts and wheat products through the Feed the Future Innovation Lab for the Reduction of Post-Harvest Losses. The project was designed to address concerns of all three ministries and to provide an example of how basic assessments of food safety could be made within the country using existing technologies.

Amanullah Alamzai, from the World Bank, followed with details of food safety interventions, both in place and projected, made by the World Bank in Afghanistan. These interventions are key to raising food safety in Afghanistan to international quality standards. Interventions may be pre- or post-harvest, and range from on-farm activities to training personnel, developing physical infrastructure, passing enforceable food safety regulations, and helping ministries other than MAIL understand their stake in the process. A part of the current infrastructure development process is to develop a lab that can be used for testing mycotoxins.

Jahed Ahadi, Director of Plant Quarantine at Kabul Customs, followed with a presentation on what MAIL had learned from the process of hosting the mycotoxin lab for these assessments. Lessons learned included: how to collect samples for mycotoxin analyses, how to screen the collected samples for toxins using commercially available kits, and how to develop and maintain a basic, functional laboratory for mycotoxin analyses. The lab established was for research and survey work only, and was not designed to be an accredited lab that could be used for regulatory purposes such as certifying goods for export or determining regulatory compliance for imports.

John Leslie, from Kansas State University, presented the overall findings of the Assessment, and described how materials had been tested in Afghanistan, at two universities in the United States, at BOKU in Vienna, Austria, and by ISPA-CNR in Bari, Italy. He described symptoms of poisoning with the mycotoxins screened, and the utility of the test kits employed for sample analysis under conditions in Afghanistan. Aflatoxins were above European acceptance levels in 15/81 almond samples, 19/40 pistachio samples, 8/25 walnut samples, and 43/89 raisin samples. Ochratoxins were above European acceptance levels in 2/40 pistachio samples and 25/80 raisin samples. Contamination levels varied widely by location sampled and variety analyzed. Different countries have different limits for these compounds in their imports, and seeking an alternative market to Europe, which has the tightest regulations in the world, could enable more exports of lightly contaminated raisins and nuts. In wheat aflatoxins were found in 23/151 samples, indicating that storage is a problem since field contamination of wheat by aflatoxin-producing species is not well known. Ochratoxin contamination of wheat, which is associated with kidney failure, occurs in Northern Europe, and was found in 36/181 samples analyzed. *Fusarium* toxins such as T-2, HT-2, deoxynivalenol and zearalenone were not found in more than a few of the wheat samples and were not a major problem in the materials analyzed. Finally, ergot alkaloids were detected in 51/153 samples assayed, and suggest a significant ergot epidemic occurred this past year in wheat, and probably other similar grasses in Afghanistan and perhaps in a broader region as well. Findings from a single year can provide a “snapshot” of a particular problem at a particular time. Variations in weather, cultivation practices and storage conditions can all impact mycotoxin contamination levels. Thus, data from any single year may indicate the potential level of contamination, but are insufficient to predict the level of contaminations in any future years.

Technical Issue Session

Asadullah Ansari, from the MAIL Plant Protection and Quarantine Laboratory, made the first presentation in this session. Samples of wheat, raisins, walnuts, pistachios and almonds were taken from Herat, Kandahar, Balkh, Samangan, Kabul, Kapisa, and Parwan, by MAIL staff. Shelled nuts, raisins and wheat (flour or grain) were taken as 1 kg samples, and unshelled nuts as 2 kg samples. Samples were taken from the market place or from storage facilities and warehouses and not directly from farmers or from the field. Storage conditions were noted and samples were taken from at least 20 bags, if samples were stored in bags, and from multiple locations within mills or storage locations if the commodity was stored as a bulk. Samples were taken in zip-lock bags and returned to the lab as soon as possible. A sample identification sheet was included in the bag with a sample to reduce labeling errors.

Mohd Rafi Rustami, from the MAIL Plant Quarantine Office, went through the methodology used for sample evaluation within the mycotoxins lab established by this project. Samples were logged into the lab’s records. Nuts were shelled before grinding. A random 300 g subsample was taken from each sample for analysis in the Kabul laboratory. Samples were processed and extracted according to the recommended procedure of the test kit manufacturer (Romer La-

boratories). The extracts were analyzed with the Romer Test kits and results recorded from a Romer StatFax 4700. Mycotoxin levels in extracts were calculated by using mycotoxin-specific Romer Log/Logit spreadsheets, which use values obtained from a dilution curve of known standards run with each set of test samples.

Luis Sabillón, a senior graduate student with Dr. Andreia Bianchini at the University of Nebraska-Lincoln, presented on potential problems in each step of the analysis process that could affect interpretation of the data. Sampling, sample preparation and sample analysis are the three main categories in which error can occur, with errors in record-keeping often imposed on top of procedural errors. Of these sampling usually is the largest source of error because mycotoxins are distributed unevenly within the sample and the average contamination level usually is low. Examples of the sources of the errors were presented as well, and included when samples were selected to look the cleanest or the most heavily contaminated. Sampling also can be a problem if an entire sample is not ground to homogeneity before being divided amongst different groups for analysis. Extraction and analytical protocols will perpetuate problems in sample collection and homogenization and introduce additional errors most commonly attributable to an incorrect manipulation of a sample or reagent. The major errors in this study appear to be in the areas of sampling (not random across the larger sample), sample preparation (not homogenizing/grinding the entire sample at one time), and record-keeping, as multiple reports of results for the same sample did not always have the same values.

Session 4 – Trade and Health Issues

Ahmed Kablan, from the Bureau of Food Security USAID/Washington, made the first presentation in this session on Mycotoxin Impacts on Child Growth and Development. In the first section of the talk he provided evidence linking childhood stunting to mycotoxin contamination, summarizing some of the literature now available to support this connection. General conditions under which mycotoxins contaminate crops were discussed and the role of Good Agricultural Practices in minimizing such contamination. He introduced the concept of Provisional Maximum Tolerable Daily Intake (PTMDI) as a more important measure for most subsistence situations, as PTMDI more accurately measures potential risk, and because diets in many developing countries are skewed heavily towards a single food source, *e.g.* maize or wheat, and regulations developed for Europe or the US even if implemented in a developing country may still lead to excessive exposure to a toxin(s) due to differences in diet composition. The mechanism by which mycotoxins result in stunting is unknown, although at least five different mechanisms are possible. Stunting is strongly associated with mycotoxin contamination, but mycotoxins alone are insufficient to account for the currently observed stunting problems. It is not uncommon for foods to be contaminated with more than one toxin, but such settings have not been examined in any way and need more attention as they could be quite important. The importance of diet diversity as a measure to reduce overall mycotoxins intake was discussed. The potential of enterosorbents, which bind toxins and prevent their adsorption in the gut, and chemopreventive agents, which prevent targeted mycotoxin activity in the body, need a great deal of additional testing to prove safety and efficacy and may be difficult to supply routinely in many of the areas in greatest need of them.

Antonio Logrieco, Director of the Italian Institute for the Science of Food Production (ISPA-CNR), discussed regulations for the import of foods potentially contaminated by mycotoxins into Europe. In recent years, 20-30% of the border rejections of contaminated foods being imported To Europe were for mycotoxin contamination. Aflatoxins and ochratoxins accounted

for > 90% of these rejections, most of which were for nuts or dried fruit from Argentina, India, Iran and Turkey. Mycotoxin regulating countries have been increasing globally since the early 1980s, and now ~100 countries regulate one or more toxins in one or more ways. Europe averages nearly 20 different mycotoxin regulations per country, nearly four times the number found in North America and 8-10 times the number found in other parts of the world. Regulations in Europe cover > 99% of the residents there and additional regulations are currently under consideration for implementation within the next few years. Globally, aflatoxin is the most frequently regulated mycotoxin followed by ochratoxin, deoxynivalenol, and T-2 toxin.

Session 7 – Food Safety and Security

Rachel Zedek, from Control Union, opened the session with a talk on food safety and international certifications. She noted that government regulations must be enforceable, but that self-regulation by private industry was a very important component of any food safety process. If the private companies do not accept responsibility for the provision of safe, wholesome food to the consumer, then no regulations will be effective. Certification is a complicated field, with >130 certification bodies occurring globally. No one lab needs certification from all of them, so selectivity is important. Implementing global certification systems is expensive, and attempts to cut corners usually fail. Once implemented successfully, however, these certifications can help develop a brand name for Afghanistan's exports that make them more valuable and ease their entry into major global markets. An important short-term plan for Afghanistan might be to pay for evaluations by certified laboratories in the private sector, perhaps outside the country, until there is sufficient capacity to staff and equip suitable laboratories within the country so that the necessary evaluations can be conducted locally. Adopting international standards and not adopting lesser standards will be important if the results are to have value and find broad acceptability. Certifications build on each other, and it is not necessary to get them all at one time, so a long-term plan for acquisition and implementation of various certifications is important. The presentation generated considerable discussion on whether the best strategy was for government labs to be operating in a certified environment or if the government would be better served by setting food safety standards and certifying labs of other entities, *e.g.*, private sector, universities or NGOs, for particular purposes/activities.

Robin Johnson, of the USAID/Kabul Communications Office, made a presentation on risk communications. How risks resulting from real or potential mycotoxin contamination are communicated within the government, business community, international trade partners and general public can make the difference between a relatively calm acceptance of a potential problem or a panic and emotion-filled response that undermines a governmental response to the problem and undercuts any work in the area for years. Science, culture, policy, relative risk, government credibility, and the means available to distribute information all play a role in determining the best way to communicate the real and potential risks to various audiences – scientific community; farmers; traders, importers and exporters; government officials and regulatory bodies; and food processors and retailers. It is important that the communication is interactive and not one way, that the information presented is credible, and that there is a clear distinction between facts and opinions. Policy and management decisions need to be flexible enough to adapt to the communication strategy and to respond to the concerns of the audience as they arise.

Ranajit Bandyopadhyay, from the International Institute of Tropical Agriculture, made a presentation on biological control of mycotoxin-producing fungi. Reducing toxin contamination pre-harvest and reducing the size of the population of mycotoxin-producing fungi is far more ef-

fective than remediating toxin contamination after it has occurred. Biological control of aflatoxin has been quite successful for maize, and that are indications that it might work well with pistachios as well. Insect control is important for aflatoxin control in pistachios as the fungus is thought to enter the nuts through holes in the shell caused by an insect. Spraying to control navel orange worm in the summer and after harvest can be very effective in reducing aflatoxin contamination of this nut. Use of aflatoxin non-producers as part of a biological control program has been effective in California. Similar strains could be developed for use in Afghanistan from local fungal populations. Strains developed for this purpose elsewhere should not be imported for use in Afghanistan. A number of potential management strategies that could be easily implemented in Afghanistan were described in the presentation as well.

John Floros, Dean of Agriculture at Kansas State University, spoke on principles of food safety and food quality in a broad sense and how mycotoxins were a part of this picture. Simple processes, *e.g.*, heating and cooling foods, are insufficient to degrade mycotoxins in most food products even though they can effectively control other types of hazards, *e.g.*, microbial contaminants. Foodborne illnesses remain a major global problem, with ~30% of the world's population affected at some time with a foodborne illness. The concept of HACCP (Hazard Analysis Critical Control Point) was introduced as a framework in which control of mycotoxins in foodstuffs should be considered. The process would begin with the selection of a variety to be planted and the management process for the plants while they were still growing in the field. Food safety is a joint responsibility of the government and the private sector.

Session 10 – Where do we go from Here?

John Leslie and **John Floros** recapped highlights from the meeting and then listed areas that were problematic and made suggestions of items and areas in which progress could be made. A major issue was confidence in the data collected. Solving this problem requires increasing the capacity for the conduct of this kind of work in Afghanistan. Both short-term and long-term (graduate degree) training are needed. The local universities must be able to train people in country to deal with issues such as those involved in basic laboratory and field operations. Short-term training can fill gaps and enable some events to occur, but should be viewed as an addition to the basic long-term training rather than a substitute for it. As there is much to be done in these areas, identifying and developing one, or at most a few, initial target areas will be critical. Facilities will remain a struggle. The buildings and equipment are deficient in one or more different ways. Maintaining facilities once they are established when basics such as electrical power and water supply are erratic is a problem. Until these problems are solved more effectively than they are now laboratories should be basic and functional, but expensive equipment, *e.g.*, HPLCs, LCs and mass spectrometers, should be avoided. More sophisticated analyses and back-up to routine work should be handled through pre-arranged agreements with laboratories outside the country. Methods to control inventory of supplies and to enable quicker processing through Customs also should be developed and implemented. Most importantly, funding sources from both the government and from multiple donors need to be identified.

There is a large web of communications that surrounds mycotoxin and food safety issues. One of the first to be solved is who is taking the lead. An overall lead is needed to coordinate efforts as are specific goals and responsibilities for different ministries, groups and individuals. A number of specific groups/individuals between whom communications on food safety need to be established were identified. Politics and people interactions also remain a potential problem at a number of different levels. Some problems include determining who gets credit for work being

done, who makes decisions on the work to be done, and who sets priorities for areas to evaluate are all structural within the government of Afghanistan. More problematic is the issue of what happens if the government changes and what is required to bring incoming high-level officials up to speed on the problem and the (re-)education of high level officials that is required to keep the process moving. Corruption can easily enter the process here and lead to certifications worth less than the paper they are printed on because the option of providing an honest “no” answer is not available. Accreditation of labs and the people involved in making such decisions is viewed as essential, but will happen only with great difficulty if fiscal corruption or threats to life and health of those running tests and communication decisions (and their families) are a reality within the country.

Drs. Leslie and Floros had some general and specific suggestions for going forward. In general, they suggested that institutions and individuals in Afghanistan become participants in the global conversation on mycotoxins, that they focus on cropping systems that have not been heavily studied in developing countries (wheat), and that they adopt management systems developed elsewhere for important crops, *e.g.*, pistachios. They also suggested some processes and protocols that could be set up: (i) Separate information gathering (research) from regulation, (ii) Begin collecting baseline data; (iii) Set up a routine process to backup unexpected or controversial results; and (iv) involve local university staff and students. Some near future actions they recommended were: (i) Short course training in various food safety principles for public and private sector employees, (ii) Address the ergot contamination problem by developing simple machinery for sorting sclerotia (which contain the alkaloids) from the wheat grain, and (iii) Identify alternative markets for nuts and raisins where the mycotoxin restrictions are not as severe as those in Europe. In the longer term, (i) effective communication strategies need to be developed to communicate the risks associated with toxin contamination, (ii) M.S. and Ph.D. level scientists need to be trained to develop local expertise, and (iii) Standards and regulations need to be developed based on a typical diet in Afghanistan and applied as widely across the country as possible, but especially to imports of staple foods.

There was a lengthy discussion by participants after a nominal group discussion section of possibilities that were proposed by the various groups. These results are summarized below in the nominal group session 11.

Nominal Group Discussions

Discussions held by the group are important for the diversity of the participants and the variation in points of view that were represented. Results are summarized by question and discussion section, and a complete set of responses and the guidelines given for the discussion process are attached. A more encompassing discussion of the results follows and contains some suggestions that could further food safety, especially with respect to mycotoxin exposure/contamination.

Technical Session Nominal Group Discussion

T-1 – Identify capacity building required for a sustainable mycotoxin surveillance program in Afghanistan.

The top two responses focus on communications and fundamental data acquisition and management. Public awareness is needed to aid data collection and a data repository is needed to discern patterns that may repeat over time and location.

The next set of responses are focused on having sufficient trained people to do the work and to be able to interpret the results obtained. Training was reflected in many responses further down the list as well, with various groups targeted for training and for particular topics that laboratory staff should be proficient to work with. “Appropriate” physical laboratories also are in this group. Identifying what an appropriate lab is varies as seen by responses further down the list, with descriptors such as accredited, quarantine, fee-based, multiple detection methods, outside Kabul, and in Kabul all included in responses.

Amongst the remaining responses that seem most significant were a need for appropriate governmental structure to deal with the issue(s), government funding for and recognition of the importance of the work, developing standard protocols to be followed, and efforts to help ensure people along the value chain from farmers to consumers were aware of issues and appropriate responses to problems that might occur.

T-2 – Identify data that should be collected to enable decisions regarding mycotoxin contamination to be made in Afghanistan.

Responses to this question often are not direct responses to the question asked, but instead are standards, protocols and processes for collecting the necessary data. Note that one response is for the use of an invalid technology for detecting toxin contamination.

More prominently mentioned data needed include GIS location, soil type, weather, variety grown, moisture content, *etc.* associated with a mycotoxin evaluation of a particular sample. Samples of data from along the value chain might help determine where mycotoxins are most likely to be increasing and to identify locations or conditions that are particularly problematic. Information on pre- and post-harvest conditions could be important, as could a more thorough evaluation of imports of potentially problematic foods.

T-3 – Identify ways to increase the credibility of the results obtained from mycotoxin surveillance surveys in Afghanistan.

Increased credibility was thought most likely to result if staff were better trained and training was an ongoing effort, and if the methodology being followed was both standardized and of international standard. There was a mixing of thoughts of whether labs had a research or a regulatory function, with public announcement of violations, enforcement of established standards, and laboratory accreditation of more importance if regulation is the lab’s function. A visible commitment from the government to the effort and the availability of data to the public were also thought to be important incentives to increase the credibility of the work conducted.

Session 6 – Nominal Group Health and Trade Issues

6.1 – Identify methods and goals for inter-ministry collaboration on problems associated with mycotoxins in Afghanistan.

At the top of this list is to define the roles and responsibilities for each ministry. Following a close second is to have regular meetings and to involve the private sector in those meetings. Clearly someone needs to own this issue and be in charge, however, and there cannot be a three-headed entity running the show. Thus, part of the definition of roles and responsibilities needs to include how the leadership issue will be managed.

Underlying the need for defined roles and responsibilities and effective communication is the development of aligned practices and guidelines. There are some specific suggestions for which ministry should be responsible for different tasks. All should have resources committed to the

effort and all should have some role in establishing guidelines, regulations, monitoring systems, mitigation practices, and outreach to those outside the government. The relationship and the activities are likely to evolve with time, so building the system with enough flexibility to allow the evolution to occur is quite important as well.

6.2 – Identify regulations needed to limit mycotoxin exposure in Afghanistan.

The most heavily weighted outcome was to establish maximum residual levels allowed in food and/or animal feed. Establishing regulations is best done by some sort of Food Safety Authority. This agency may need to be independent of the three ministries but have reporting responsibilities to all of them. Certainly coordination amongst the ministries and the Food Safety Authority will be essential. This agency could then be authorized to establish guidelines within various parameters, and could adapt guidelines and regulations as new information became available rather than waiting for legislative decisions on technical matters. Delegating responsibility for Food Safety issues that extend beyond mycotoxins should be a relatively straightforward process.

Responses past these initial high-ranked responses scattered in many directions, including particular places and situations where regulations should be enforced, how domestic and imported items should be treated, inspection processes for public and private labs, development of SOPs that go from farmers through to consumers, working conditions (especially security) for inspectors and other potentially targeted individuals in the regulatory process, and where the funding for the work to be conducted will come from.

6.3 – Identify cultural barriers to be overcome to reduce mycotoxin exposure to mycotoxins in Afghanistan.

Responses to this question indicate that a significant study of how foodstuffs are managed by various groups in the country is going to be needed to help any proposed interventions succeed. Changes to traditional agricultural processes, food processing and food storage practices will be especially important to implement in a careful and thoughtful manner. Dietary changes that reduce dependence on wheat as a staple food also may be difficult.

Beyond these major points many of the issues encountered may result from limited education of farmers and rural women who are responsible for much of the crop cultivation, food storage and food processing. Ensuring that communications come to them from a trusted authority is important and may be difficult to achieve.

6.4 – Identify benefits resulting from lesser exposure to mycotoxins in Afghanistan.

The two top benefits identified were improved health and improved economic growth with more jobs. Perceived health benefits were reduced morbidity and mortality, less childhood stunting, improved productivity (as workers would not be out sick as often), and reduced costs from sending people outside the country for medical treatment. Increased health of domesticated animals could increase the availability of meat and other animal products as foods in the domestic markets.

Perceived economic benefits were quite numerous and most were not widely supported. They ranged from more food of better quality available in local markets to higher incomes for everyone along the value chain, and a better reputation (and price) for exports from Afghanistan with fewer rejections of exports as substandard. The ability for government ministries to work with each other and with private sector to reduce the problem would provide evidence that the

government was doing something positive for the people and could open the doors to additional joint activities. A success of this sort would lift morale of many of those working in the food production business.

Session 8 – Nominal Group Food Safety and Security

8.1A – Who needs information on mycotoxins in Afghanistan?

The basic answer to this question was everyone. At the top of the list were farmers, consumers, traders regulatory officials and extension workers. Some less obvious choices included on the list were health care providers, veterinary clinic staff and religious leaders. This question and question 8.2B are the only ones where every response was on at least one individual's "Top Five" list.

8.1B – How should information on mycotoxins in Afghanistan be delivered?

There are many ways that information on mycotoxins could be delivered. The top three were public media (radio, TV, print, *etc.*), official government publications, and social media. These methods seem targeted at the broad consuming population as a whole. The remaining suggestions begin to fragment the population, with workshops and extension personnel ranking next. MAIL was the only ministry identified as needing to provide information, and that responsibility probably should be spread over all three ministries, but with differing target audiences.

8.2A – When should screening for mycotoxins occur in Afghanistan?

Screening was envisioned as a routine thing for all commodities, with only one response suggesting that screening should be determined on the basis of environmental conditions. All but two responses suggested that screening should occur at harvest time or later, with processing, storage, market place and prior to export all receiving relatively strong support. Screening of materials to be imported was not ranked particularly highly.

8.2B – Where should screening for mycotoxins occur in Afghanistan?

Some responses to this question are quite distinct from those to the "when" question (8.2A). The two most prominent locations were in the field prior to harvest and for imports at the border, followed by the marketplace and at the borders. Again there were a couple of responses suggesting that testing was needed at some times and not others, *e.g.*, "suspected locations" and "for crops at highest risk".

Session 11 – The Future

For questions 11.1A and 11.1B, participants were asked to mark their top seven choices, instead of the top five, as was done with the other questions.

11.1A – Identify priorities for the next year for research on mycotoxins and potential applications of solutions in Afghanistan.

Three of the top four priorities focus on government actions that can be started without significant scientific efforts. In particular, to establish an inter-ministerial/private sector task force (with a defined agenda and distributed responsibilities), to begin work to disseminate information to the general population, and to identify budget funds and show a commitment to work on mycotoxin reduction. Continuing the mycotoxin survey begun by this project was the fourth of the top priorities.

Education for MAIL staff and for exporters were the next most strongly supported activities. As with the first four activities, these activities could be seen as preparing groundwork for larger efforts in the future.

The remaining responses were quite scattered, and probably indicate the number of different directions that the work could take. I list below some of the ideas that seemed potentially the easiest to implement and where impact might easily be seen relatively quickly:

- Identify donors and other stakeholders and begin conversations with government ministries and private sector.
- Develop Good Agricultural Practices for Pre- and Post-Harvest management of crops.
- Begin analysis of value chains so that Critical Control Points in the HACCP process can be identified.
- Finalize food safety law and develop a series of SOPs for its implementation, including adopting limits on the most important mycotoxins.
- Adapt manuals (http://www.calpistachioresearch.org/GAP_Manual_2009.pdf) from the California Pistachio Research Board for local use. The main focus is on preventing fungal infections and subsequent mycotoxin contamination. There are numerous additional potentially useful links from the CPRB's Home page that could be modified for use in Afghanistan. Some information already available at: http://afghanag.ucdavis.edu/a_horticulture
- Adapt GAP guidelines from the California Almond Board (<http://www.almonds.com/growers/growing-safe-product/gaps#harvest-delivery-sanitation>) for local use. These guidelines suggest food safety practices that extend far beyond concerns regarding mycotoxins. Some information already available at: http://afghanag.ucdavis.edu/a_horticulture. Similar information can be found for walnuts at: <http://www.walnuts.org/>. Information of this sort for raisin production in Afghanistan is already available on line (http://afghanag.ucdavis.edu/a_horticulture/fruits-trees/grapes).

11.1B – Identify priorities for the next 5-10 years for research on mycotoxins and potential applications of solutions in Afghanistan.

Responses in this section are a continuation of those from the previous question. Many of the responses implicitly assume that many responses to 11.1A have been accomplished. Some responses are for continuation of these efforts, for instance Human and Institutional Capacity Development, the number 2 response, is going to be an ongoing process as will work on GAP and HACCP processes and protocols. By this time government funding should be firmly committed to the work, regulatory standards should be established, a functional Food Safety Authority should be in place, and the inter-ministry/private sector working group should be a routine activity.

Challenges awaiting this time frame are the accreditation process for public and private laboratories, a decision on whether the government should be involved in “certifying” exports, enforcement processes for border inspections should be established, and at least some training of personnel to work in the area will be conducted by local experts. SOPs should be in place all along the value chains for the toxins relevant to those value chains, and a series of regional labs to provide quick tests should be in place around the country. If surveys have been conducted on an annual basis, then there should now be enough data to determine if there are any crop/geographic/weather hotspots for toxin occurrence patterns to be discernable.

Research questions will focus on agronomic and storage practices to reduce contamination, methods of mitigating contamination once it has occurred, and uses other than human food for materials contaminated with high levels of mycotoxins.

Awareness

Awareness of mycotoxins and their potential impact in trade, agriculture and health was a theme that echoed in all of the nominal group discussions of both the technical and the more diverse nominal group sessions. Awareness comes in a number of different forms and formats and needs to be distributed all along the value chain from farmer to consumer. The needs of the employees of ministries who help manage the problem are different from those of traders and private sector actors who are buying/selling and importing/exporting agricultural goods which are different again from university staff/students conducting research in this area and differs even further from that of the farmers or the general population in the city and in rural areas. Raising awareness is critical and must be done in a manner such that those who hear the message are energized to address the problem in a positive manner and not are so frightened that they freeze up and cannot do anything.

Public awareness was a major theme and one that requires care in developing. Afghanistan does not have a history of deaths or other severe debilitations tied explicitly to a mycotoxin, *e.g.*, aflatoxins in Kenya or fumonisins in South Africa. The approach at this time should be towards better post-harvest storage practices, increased food quality, and care and cleanliness in food preparation.

MAIL, MoPH and MITC will need to collaborate to establish a common theme and priorities. MAIL should be talking to farmers, MAIL and MITC need to jointly talk to traders and importers/exporters, and MoPH and MAIL should be talking to consumers and the general public. Such efforts require commitment from the top to the task and buy-in from those working in the middle levels of the ministries if the desired outcome is to occur. An important first effort will be to have training sessions on the inter-relatedness of the issues for ministry staff. The training could take many different forms, but the most important will be enough team-building to have staff from all three ministries talking about collaborative, rather than competitive, approaches, solutions and endpoints. USAID and other external players may need to assist with this training as the number and depth of trained personnel available within the Government of Afghanistan is very limited.

Farmers need to understand their role as conditions before and during harvest can have a major impact on the amount of mycotoxin contamination in items entering the food system. Training in Good Agricultural Practices is the single most important thing that could be done to reduce mycotoxin contamination in Afghanistan. Incorporating background information on the detrimental effect of these compounds into the GAP training is probably the easiest way to get this information to farmers. GAP training can occur in many different ways. SWABO (Scientific Animations Without Borders), through the Post Harvest Loss Innovation Lab, has developed numerous cell-phone based training modules and games that have been well received in other developing countries and have been used successfully in conjunction with more traditional outreach programs.

Traders and importers/exporters need to know that mycotoxins can reduce the value of the items they are buying and selling. In the case of exports, mycotoxin contamination can not only affect the price, but also may affect whether a product can be sold at all, or must be destroyed at the exporter's expense. That different export markets have different sensitivities to mycotoxin contamination needs to be more generally known and could open up new markets that could be more easily penetrated than those of the European Union, whose regulations are the strictest in the world.

Capacity

Afghanistan needs to develop the capacity to manage mycotoxin contamination locally. Physical and human capacity both are currently limiting. Physical capacity includes appropriately equipped laboratories with 24-hour electricity and secure storage for reagents and samples, as well as appropriate means for disposing of contaminated samples and hazardous materials generated during the analytical process. Human capacity requires staff with both specific training in particular activities and general training in mycotoxins and associated activities.

Once basic human and physical capacity needs are met, then capacity for doing the work can be assessed. Appropriate SOPs for the analysis(es) being conducted must be developed and implemented, and a process to validate results and estimate errors established. External assessment of the lab's capacity also must be conducted in a manner that honestly evaluates the credibility of the results reported. Developing credible laboratory capacity for research and information purposes should be possible in governmental, university and private settings.

Developing credible laboratory capacity that could be used for regulatory purposes might be possible for a private laboratory, but the culture of power and corruption associated with government agencies will make developing credible regulatory capacity much more difficult in a government setting. For regulatory purposes, a better approach would be to develop the capacity to accredit laboratories, rather than to simply have laboratories in which work is conducted be effectively accredited simply because they are government run. The capacity to accredit laboratories for their ability to assess food safety could be extended far beyond mycotoxin analyses and would be a significant government service for the country as a whole.

Medical assessments

Public health measures *per se* were not the major topic of this conference, but are an important component of addressing mycotoxin contamination problems. The extent to which individuals have been exposed to various toxins is important to understanding the mitigation steps that should be taken. Biomarker assays using both blood and urine are becoming available for many toxins. These protocols require medically trained personnel to conduct studies as part of an interdisciplinary team looking at the overall food availability and food security problems in the country.

Beyond Mycotoxins

Much can be done in terms of food safety that goes beyond mycotoxins. Both chemical and biological, primarily microbiological, hazards exist. Including mycotoxin work within this broader food safety context probably is essential for sustainable research and regulation of mycotoxins.

A second area worthy of further research is the effect of fungal secondary metabolites beyond mycotoxins on human and animal health. There are numerous secondary metabolites that are not toxic in and of themselves, but certainly can impact human health. In this survey citrinin and mycophenolic acid were detected and these compounds can alter immune system activity and kidney function, respectively. Some of the unknown causes of these problems may be related to synergistic interactions with mycotoxins or other secondary metabolites. Little work is done in this area, and could be very important as the emphasis of research shifts from acute mycotoxicoses to assessing the results of chronic exposure to contaminated foodstuffs.



International Conference on Food Quality and Safety Creating a shared vision and partnership

DATE: 14-16 March, 2016, 2016

LOCATION: The Imperial Hotel – New Delhi, India

OBJECTIVES:

- a) Review and discuss findings of “Rapid Assessment of Mycotoxins in Afghanistan’s Food Value Chains”
- b) Discuss implications of this assessment as it pertains to regional trade, exports, food security and human health
- c) Devise a basic Action Plan for policy reforms, private sector engagement and donor coordination

14 March 2016

Time	Topics	Presenter/ Facilitator
08:30 – 09:00	USAID India Welcome – Ambassador Jonathan Addleton USAID Afghanistan Welcome Introduction of participants Review of Agenda	McDonald Homer, OAG Deputy Director
09:00 – 10:00	Group Session 1: Introductory Remarks from Heads of Delegations <ul style="list-style-type: none">▪ Ahmed Kablan, USAID/Bureau of Food Security (BFS)▪ Minister Feroz, Ministry of Public Health▪ Mohammad Anwari, Ministry of Commerce & Industry▪ Deputy Minister Haidari, Ministry of Agriculture, Irrigation & Livestock▪ Dean John Floros, Kansas State University	McDonald Homer
10:00 – 10:15	Group Session 2: Nominal Group Discussion Setup	John Leslie
10:15 – 10:45	Tea Break	
10:45 – 12:15	Session 3: Summary of Mycotoxin Assessment; Q & A <ul style="list-style-type: none">▪ Origin of the Assessment – McDonald Homer▪ World Bank – Amanullah Alamzai▪ What We Learned – Jahed Ahadi▪ Overview of Results – John Leslie	John Floros
12:15 – 13:30	Lunch at Imperial Hotel	

14 March 2016 Policy Group Schedule

Time	Topics	Presenter/ Facilitator
14:00 – 15:00	Site Visit A: Food Safety & Standards Authority http://www.fssai.gov.in/ FDA Bhawan near Bal Bhavan, Kotla Road	McDonald Homer
15:30 – 16:30	Site Visit B: Presentation by CHAMP team at Imperial Hotel http://rootsofpeace.org/usaids-champ_india_office_ribbon_cutting_175x_150_q50	H. Hamid Safi, USAID/OAG
17:00 – 18:00		
18:00 – 19:00	Group Session 4: Trade and Health Issues <ul style="list-style-type: none"> ▪ Mycotoxins & Health – Ahmed Kablan ▪ Mycotoxin Regulations – Antonio Logrieco 	John Floros
19:30	Dinner at Imperial Hotel	

14 March 2016 Technical Group Schedule

Time	Topics	Presenter/ Facilitator
13:30-14:45	Technical Issue Presentations <ul style="list-style-type: none"> ▪ Afghanistan – Sample Collection Protocols – Assadulah Ansari ▪ Afghanistan – Laboratory Protocols & Data Collection – M. Rafi Rustami ▪ Data from outside Afghanistan and total data analysis/synthesis – Luis Sabillon 	John Leslie
14:45 – 16:30	Technical Nominal Group Discussions <ul style="list-style-type: none"> ▪ Identify capacity building required for a sustainable mycotoxin surveillance program ▪ Identify data to be collected for making decisions on mycotoxins in Afghanistan ▪ Identify ways to increase the credibility of the results obtained in Afghanistan 	John Leslie
16:30 – 17:00	Tea Break (after finishing first or second question)	
17:00 – 18:00	Technical Nominal Group Discussions (continued)	John Leslie
18:00 – 19:00	Group Session 4: Trade and Health Issues <ul style="list-style-type: none"> ▪ Mycotoxins & Health – Ahmed Kablan ▪ Mycotoxin Regulations – Antonio Logrieco 	John Floros
19:30	Dinner at Imperial Hotel	

15 March 2016 Combined Group Schedule

Time	Topics	Presenter/ Facilitator
06:30 – 08:00	Breakfast at Imperial Hotel	
08:00 – 08:30	Group Session 5: Previous Day Recap and depart for Site Visits	John Floros John Leslie
09:30 – 11:00	Site Visit C: Food Testing Lab (Technical Group) 373, Udyog Vihar, Phase II, Section 20, Gurgaon-122016, Haryana	John Floros
09:30 – 11:00	Site Visit D: Airport Cargo Section (Policy Group)	MacDonald Homer
12:00 – 13:30	Lunch at Imperial Hotel	
13:30 – 15:30	Group Session 6: Nominal Groups – Trade & Health Issues <ul style="list-style-type: none"> ▪ Inter-ministry collaboration and goals for mycotoxins ▪ Regulations needed in Afghanistan ▪ Cultural barriers to be overcome ▪ Returns from lower levels of contamination 	John Leslie
15:30 – 15:45	Tea Break	
15:45 – 17:30	Group Session 7: Food Safety & Security Going Forward <ul style="list-style-type: none"> ▪ Comments from Control Union – Rachel Zedeck ▪ Risk Communication – Robin Johnson ▪ Preharvest mycotoxin control – Ranajit Bandyopadhyay ▪ Food safety beyond mycotoxins – John Floros 	Frida Bwenge
17:30 – 18:30	Group Session 8: Nominal Groups – Food Safety & Security <ul style="list-style-type: none"> ▪ Who needs information on mycotoxins? How should it be delivered? ▪ When/where should screening for mycotoxins occur? 	John Leslie
19:00 – 20:30	Reception followed by Dinner <ul style="list-style-type: none"> • Afghanistan Embassy to India • International Airport • Ministry of Women & Child Development • Food Safety & Standards Authority • USAID/India • CHAMP/India 	

16 March 2016 Combined Group Schedule

Time	Topics	Presenter/ Facilitator
06:30 – 08:30	Breakfast at Imperial Hotel	
08:30 – 08:45	Group Session 9: Previous day Recap	John Floros
08:45 – 09:30	Group Session 10: Where do we go from here? <ul style="list-style-type: none"> ▪ Possible responses to the problem – John Leslie & John Floros 	Ahmed Kablan
09:30 – 11:00	Group Session 11: Nominal Group Session – The Future <ul style="list-style-type: none"> ▪ Public/Private/Donor sector coordination ▪ Sustainability of World Bank sponsored and other labs under the care of the Government of Afghanistan ▪ Priorities for next year and next 5-10 years – research and implementation 	John Leslie
11:00 – 11:30	Group Session 12: Final Remarks/Group Photo	McDonald Homer
11:30	Airport departure for 14:30 flight to Kabul	



The College of Agriculture at Kansas State University

**Presented at the USAID Conference on
Food Quality and Safety**

Delhi, India, March 13-15, 2016

**John D. Floros, PhD
Professor of Food Science & Engineering
Dean of Agriculture & Director of KSRE
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THROUGH INTEGRATED RESEARCH, ANALYSIS AND EDUCATION



OUR VISION:

A **top five** college of agriculture

A **global destination** for education, research, and extension



In Kansas

AGRICULTURE LEADS

**\$62.8
BILLION**

43% of the
economy — state's
largest industry

**229,000
EMPLOYEES**

State's
largest
employer

**\$4.9
BILLION**

Goods exported
— state's largest
exporter

**\$26.9
BILLION**

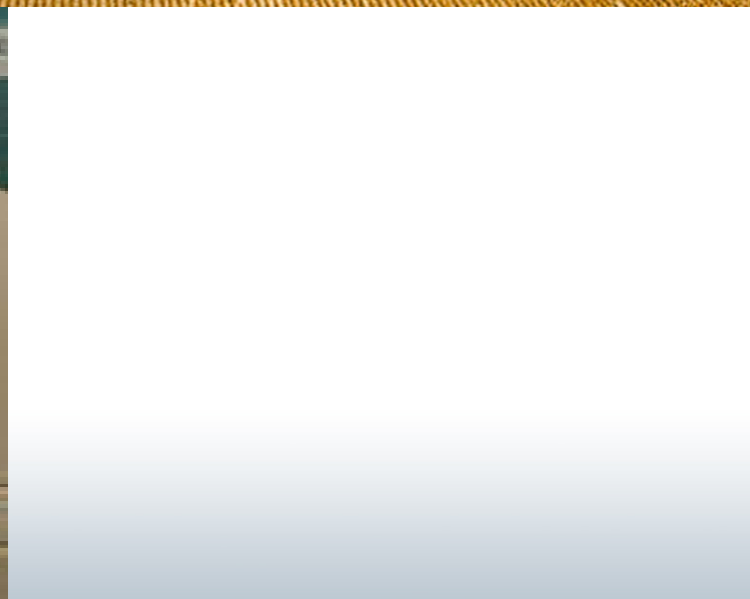
Crops and livestock
total economic
impact for Kansas

**46,137,295
ACRES**

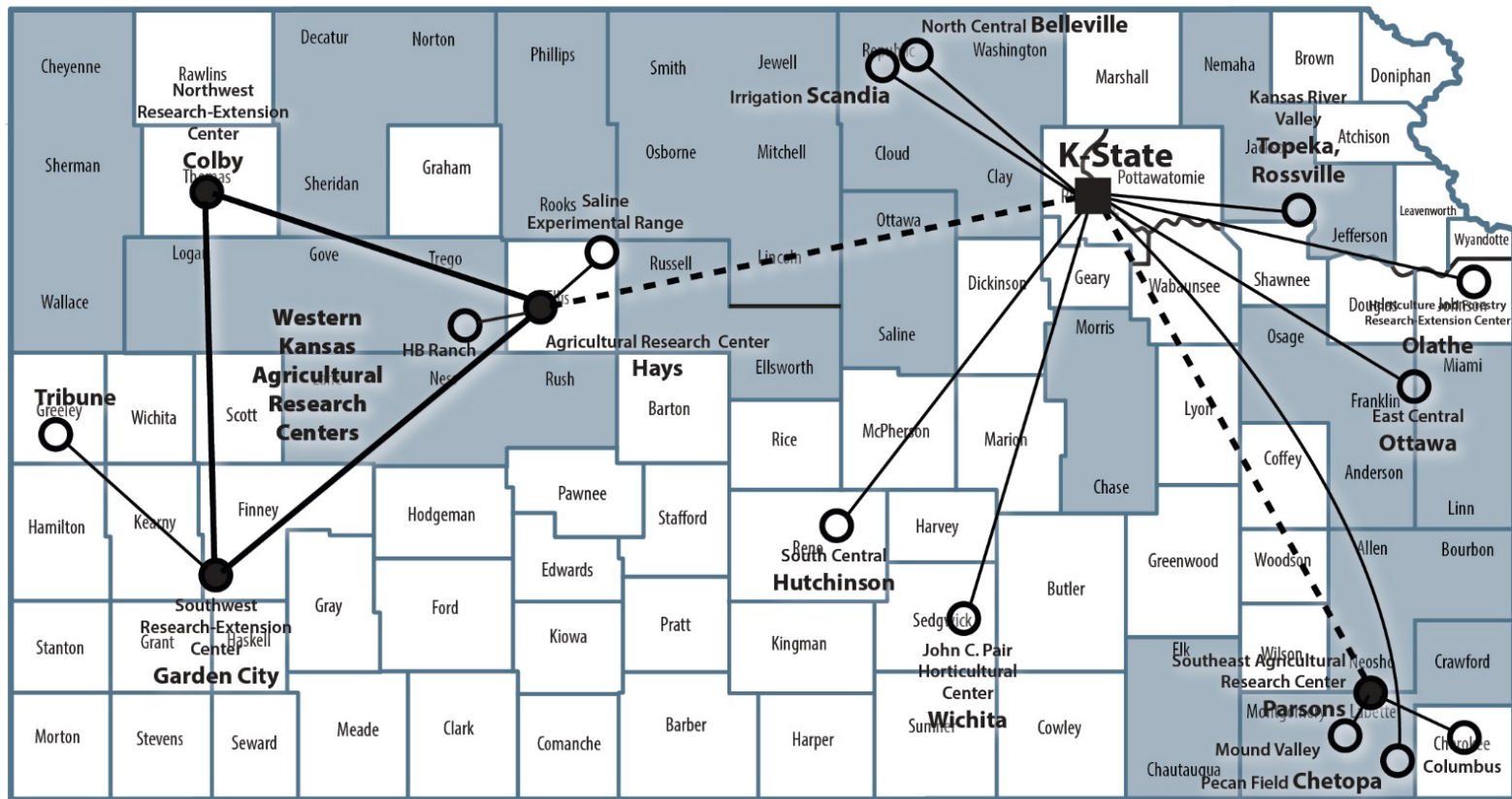
Farmland in
Kansas —
88.9% of all
Kansas land

**8
COUNTIES**

Dependent on the
Ogallala Aquifer
produce 1/3 of the state's
agriculture revenue



SERVING THE ENTIRE STATE OF KANSAS





GRAND CHALLENGES



GLOBAL FOOD SYSTEMS

K-State Research and Extension plays a vital role in preparing to feed the 9.6 billion world population expected by 2050 through modern technology and techniques utilizing:

CROPS

- Wheat
- Sorghum
- Corn
- Soybean
- Canola

LIVESTOCK

- Beef cattle
- Dairy cattle
- Swine
- Poultry
- Sheep/Goats

VALUE ADDED

- Food processing
 - Meat
 - Dairy
 - Milling
 - Baking
 - Extrusion
- Food Quality & Safety
- Feed Processing
- Ethanol Production



DOCTORAL PROGRAM RANKINGS IN THE US



No.1

PLANT PATHOLOGY
DEPARTMENT



No.4

AGRICULTURAL
ECONOMICS PROGRAM



No.5

ANIMAL SCIENCE DOCTORAL
PROGRAM FOR RESEARCH
PRODUCTIVITY



No.8

ENTOMOLOGY
DOCTORAL PROGRAM



No.9

INTERDEPARTMENTAL
FOOD SCIENCE PROGRAM



No.10

PLANT SCIENCES

The only Grain Science Program in the USA

Dr. BIKRAM GILL IN NATIONAL GEOGRAPHIC





#1 KANSAS WHEAT VARIETIES IN 2015 HARD RED AND HARD WHITE WINTER



FUNGAL GENETICS STOCK CENTER

Will help our breeding programs be more successful

- World famous center



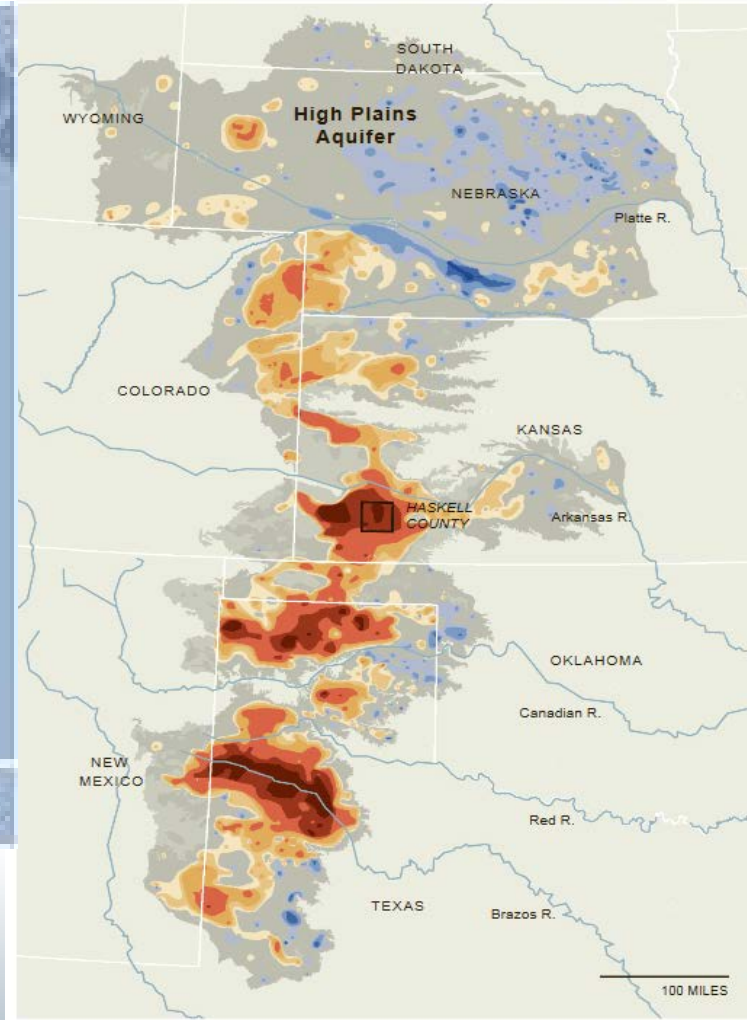
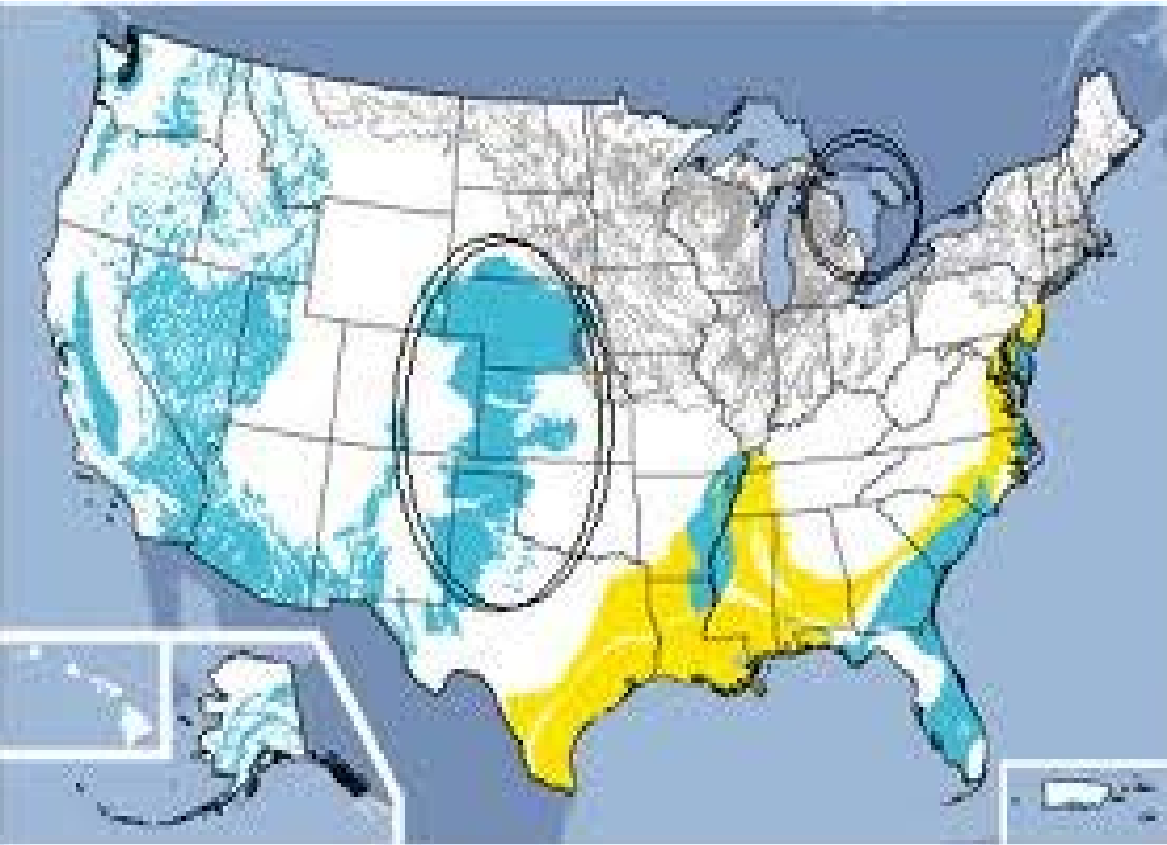
Water



**Over 2.5 Trillion
M³ of water is
consumed by
the global
agricultural
sector each year**



Ogallala Aquifer – An Underground Pool Drying Up







RECOGNIZING EXCELLENCE

We received four U.S. Agency for International Development Feed the Future Innovation Labs (**\$102.2 Million**):

- Sorghum and Millet (**\$23.7 Million**)
- Applied Wheat Genomics (**\$5.0 Million**)
- Postharvest Loss Reduction (**\$23.5 Million**)
- Sustainable Intensification (**\$50.0 Million**)

It is estimated that 30–50% (or ~2 Billion tones) of all food produced on the planet is lost before reaching a human stomach



In South East Asian countries, losses of rice can range from 37–80% of the entire production



**In India, 21 M Ton
of wheat is
wasted each year
due to
inadequate
storage and
distribution
systems**



**Improved
harvesting
systems in
developing
nations must
be supported
by efficient
storage,
processing and
distribution
systems**





Questions?

KANSAS STATE
UNIVERSITY



Outreach. Our Mission Director emphasized the importance of being pro-active in broadening our engagement with the stakeholders and possible partners. The steps taken in India for STIP programming can serve as valuable lessons for efforts in Afghanistan.

“Think outside the box”. Bill also stressed that STIP requires us to take bold, innovative steps in addressing Afghanistan’s development needs.

An appropriate technology approach. The technology employed does not have to be high-tech. Adoption of simple techniques (e.g. use of raised beds in agriculture, properly drying grains and legumes in order to reduce Alfatoxin) can also have a great and sustainable impact.

Office of Acquisition and Assistance’s involvement. To move STIP interventions from the concept phase to reality requires getting mechanisms approved and funded. This has been a bottleneck; consequently, it was suggested that OAA have a representative on the Working Group.

MYCOTOXIN = "FUNGUS" + "POISON"
A THREAT TO FOOD AND FEED

Natural toxic substances produced by fungi

KATELYN WILL YERD.COM

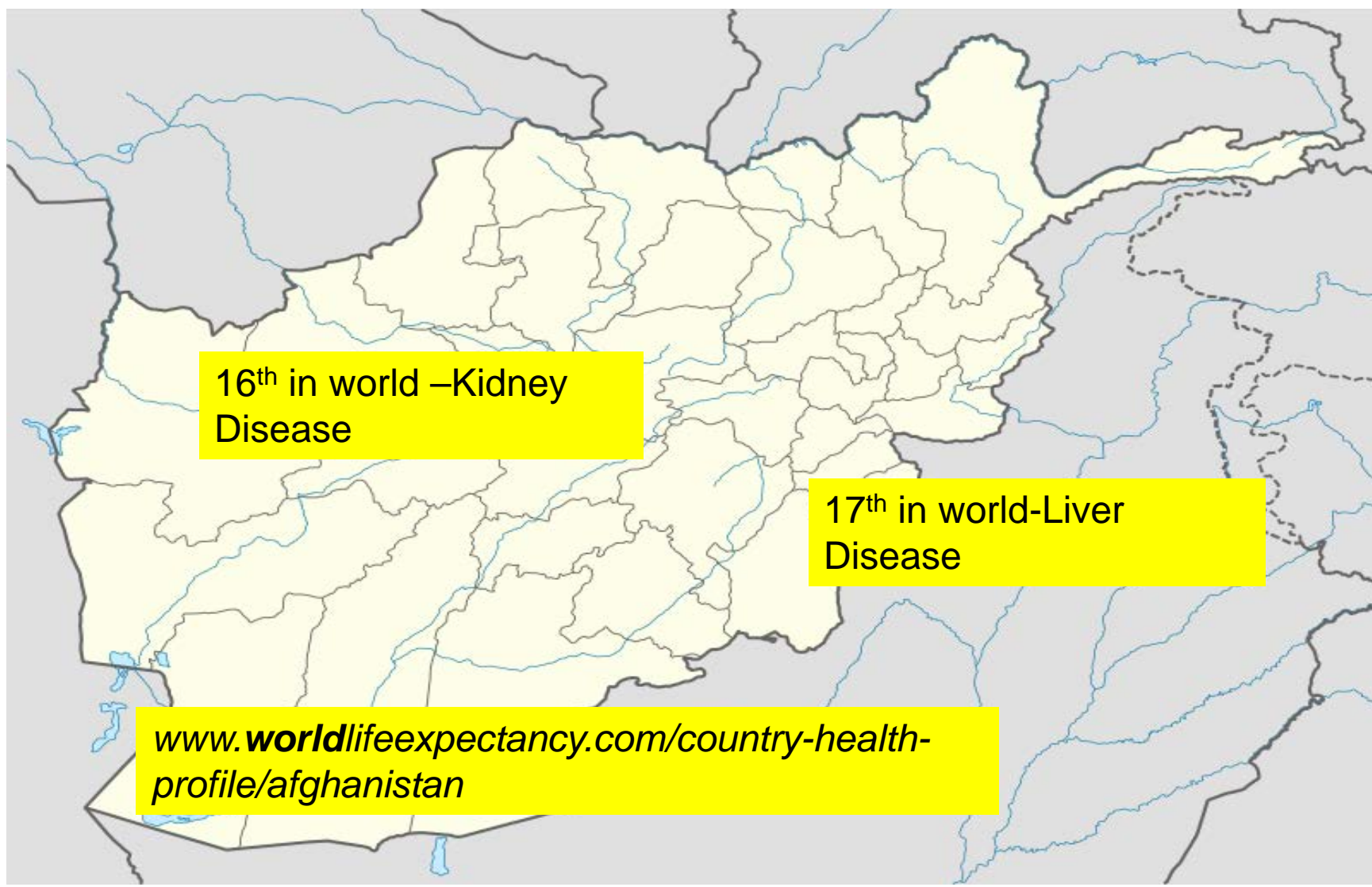
Mycotoxins in Food

Affects a wide range of food; grains, vegetables, livestock food chains



Myco=fungi; various types of fungi—different toxins, e.g. Aflatoxins, Fumonsisins

- Kidney failure
- Liver cancer
- Growth Stunting
- Immune deficiency or suppression
- Financial losses
- Nutritional Impact



16th in world –Kidney Disease

17th in world-Liver Disease

www.worldlifeexpectancy.com/country-health-profile/afghanistan



EUROPEAN COMMISSION
HEALTH & CONSUMERS DIRECTORATE-GENERAL

Directorate G – Veterinary and International affairs
G4 – Food, Alert system and training



Brussels, 4 March, 2014

MAIL seeking technical assistance to build capacity to monitor and address mycotoxins in horticultural exports



FOOD
INFORMATION EXCHANGE

BORDER REJECTION NOTIFICATION: 2014.AKL
ORIGINAL NOTIFICATION

SUBJECT: AFLATOXINS IN PISTACHIO KERNELS FROM AFGHANISTAN, VIA TURKEY



Development Challenge:

**Is there a prevalence of mycotoxins in
Afghanistan's food value chain?**



Why conduct the assessment?

- Ascertain the scope of the problem---not an *ad hoc* approach but proven, science-based methodology.
- Understanding the scope of the problem helps with devising the most effective solutions.
- Gateway activity to other work in the areas of: food quality/safety, post-harvest loss mitigation.
- Sets example of how science & technology applications can be used to address practical problems.



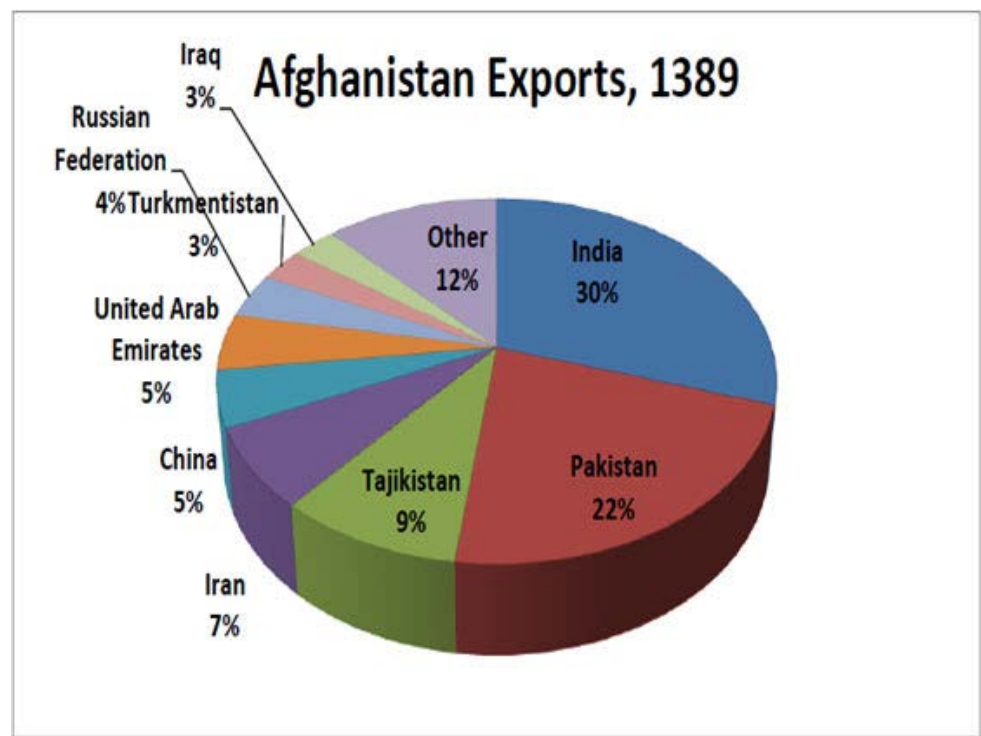
Assessment components:

- Assessment
- Technology Transfer (field test kits, ELISA)
- Technical Capacity Enhancement (MAIL)
- Equipment/Supplies
- Information Dissemination
- International Conference
- Public-Private Partnerships
- USAID review/application of findings for current and new programs



The Feed the Future Innovation Lab for the Reduction of Post-Harvest Loss (Kansas State University) is a strategic and applied research and education program aimed at providing global leadership in food security by reducing post-harvest loss and food waste of durable staple crops (grains, oilseeds, legumes, root crops, seeds) and their processed value-added products.







USAID
FROM THE AMERICAN PEOPLE

AFGHANISTAN

- Valuable Contributions for Investment:
 - Increase horticulture exports
 - Improve health/nutrition
 - Improve coordination public/private sectors and actors & donors
 - Foundation for engagement for Indian private and public sectors

Food Safety Conference
New Delhi, India
March 14-16, 2016



Why is food safety important to our work?

- More stricter rules on import of food stuff.
- The vast majority of consumers now place growing emphasis on food quality and safety.
- Unsafe and poor quality food causes.
 - 1) Health, nutrition, growth disorders
 - 2) Acute and chronic diseases, death
 - 3) Pressure on health care
 - 4) Welfare and society loss of manpower
 - 5) Food loss – food insecurity

Key hazards include:

- a. Biological: infectious micro-organisms (E. Coli, Salmonella)
- b. Chemical: Residuos (pesticides, medicines, hormones)
- c. Physical: Sand, wood, glass, metal, etc



- Quality gap (between what is produced and demanded mainly when it comes to higher paying international markets, e.g. high levels of ochratoxin in rains)
- Thus, producers/processors must adopt strict production standards to comply with the emerging quality requirements.
- Needs must be addressed through research, education, and value addition.

World Bank Interventions in Afghanistan

1. National Horticulture and Livestock Project

- Budget : USD 100 million with additional USD 90 million to be added by end of April 2016, (USD 28 million beneficiaries contribution) total USD 2018 M
- Closing date : December 31, 2020
- Coverage: 26 provinces (to be expanded to 34 provinces, if security allows)



World Bank interventions in Afghanistan cont.

- The Project Development Objective (PDO): To promote adoption of improved practices.(pre and postharvest)
- Has reached over 200,000 beneficiaries with:
 - ❑ Improved perennial crops and vegetables varieties (over 8, 000 hectars of new commercial orchards have been establishment with intercropping)
 - ❑ Improved technologies/practices for rehabilitation of existing orchards (over 75,000 hectars)
 - ❑ Support in application of IPM through facilitation of Farmer Field School(FFS).
 - Biological control (Bacteria, fungus and Viruses)
 - e.g. Trichogramma for control of apple codling moth.
 - The agents are produced in house.



- Use of environmentally friendly approaches such as vermicomposting , protective bagging, and bio-pesticides for safe and sustainable production.
- Transfer of knowledge to male and female farmers through digital audio ,video clips and face to face interactions.
- Established improved raisins production facilities; over 350 improved houses have been built.
- Improved post-harvest tools.
- Milk Hygiene : Clean and disease free milk.
- Safe and quality meat through vaccination.



2. Afghanistan Agricultural Inputs Project

- The Project Development Objective (PDO): Strengthened Institutional Capacity and Reliability of Agricultural Inputs and Sustainable Production of Certified Wheat Seed. (USD 74.75 Million). Main components include:
 - ❑ Development of commercially viable seed sector
 - ❑ Agrochemicals: Improving safety and reliability of agricultural inputs.
 - ❑ Input Delivery System

Key activities- Agrochemicals Component

- Equipment, instruments and chemicals support to PPQD, KU, ARIA, MoPH
- Nation wide pest and disease survey
- Plant and animal quarantine network and border stations
- Establishment of Pesticides Poisoning Management Centre (PPMC)
- Capacity enhancement of MAIL personnel (short term trainings and Masters and PhD programs.
- Development of relevant Laws (pesticides, fertilizers, plant protection and quarantine)

Establishment of lab complex

I. Fertilizer and Pesticide Testing Component (FPTC)

- 1) Pesticide Quality Control Lab
- 2) Pesticide Formulation Analysis Lab
- 3) Fertilizer Quality Control Lab
- 4) Fertilizer Formulation Analysis Lab
- 5) Pesticide Residue Analysis Lab

II. Agri. Products Quality Testing Component (APQTC)

- 1) Heavy Metals Testing Lab
- 2) Aflatoxin Testing Lab
- 3) Microbes Testing Lab

III. Plant Pest and Disease Diagnosis Complex (PPDDC)

- 1) Entomology Lab
- 2) Virology Lab
- 3) Bacteriology Lab
- 4) Mycology Lab
- 5) Nematodes Lab



3. **Food for All Strategic Partnership (Netherlands and the World Bank Group)**

- ▶ Creating leverage to transform the agricultural sector by mobilizing knowledge and innovation.
- ▶ World Bank and IFC: use Dutch knowledge and know-how on multi-stakeholder partnership approach.
- ▶ The Netherlands: use the World Bank Group's leverage and scaling capacity.
- ▶ Focusing collaboration in 3 key strategic areas:
 - I. Food safety and health (nutrition sensitive agriculture)
 - II. Inclusive and sustainable agricultural growth (value chains and market transformations)
 - III. Ecologically sustainable food systems (climate smart and resilient agriculture)

Thank You!



EAT

The word "EAT" is constructed from a variety of fresh produce. The letter 'E' features a banana, kiwi slices, grapes, and red peppers. The letter 'A' is filled with kiwis, grapes, and red peppers. The letter 'T' includes kiwis, grapes, and red peppers.

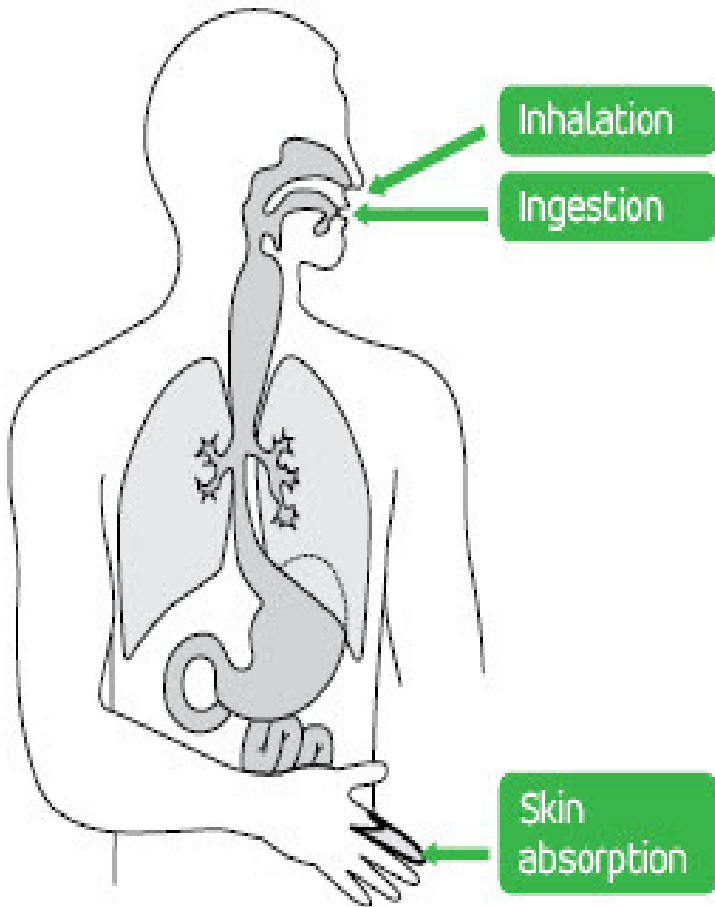
CLEAN

The word "CLEAN" is constructed from a variety of fresh produce. The letter 'C' features kiwis, grapes, and red peppers. The letter 'L' includes kiwis, grapes, and red peppers. The letter 'E' is filled with kiwis, grapes, and red peppers. The letter 'A' includes kiwis, grapes, and red peppers. The letter 'N' features kiwis, grapes, and red peppers.

Mycotoxin impact on

- Public Health
- Plants , plant products (export &import)

Routes of Exposure



Mycotoxin challenge – Afghan Plant Products

- MAIL, No facilities (or lab equipment for mycotoxin analyses)
- No capacity or skill for detection of mycotoxins.
- PPQD, the with support of USAID, began sample collections in seven provinces (Kabul , Herat , Kandahar, Balkh , Parwan, Samangan and sur-e-pul) over 700 samples (Almonds ,Pistachios, Walnuts ,Raisins Wheat and flour)
- Over 500 samples run in PPQD lab.
- Most samples sent to Austria for detailed chemical mycotoxin analyses and to the US and Italy for verification.



Project Benefits

- Capacity building.

Trained 12 PPQD staff members.

Mobilized and equipped the Mycotoxin lab in PPQD.

Now can detect several mycotaxins in plant products.

Need to obtain ISO accreditation to be able issue certificates.

- For sustainability

MAIL (PPQD) staff need more training and lab equipment for central and border labs.

Lab - Mycotoxins Analyzed

- 1- Aflatoxins – *Aspergillus* spp.
- 2- Ochratoxin – *Aspergillus* spp.
- 3- T-2 – *Fusarium* spp.
- 4- Deoxynivalenol (DON) – *Fusarium* spp.

Income – Raisins and Nuts

Afghanistan exports Raisins & Nuts to over 50 countries

2014 (CSO)			
NO	Product	Quantity(Tons)	Cost (US\$)
1	Red Raisins	1,012,871	19,244,563
2	Green Raisins	758	1,667,705
3	Black Raisins	3,716	5,203,751
4	Golden Raisins (Abjosh)	4,525	11,260,397
Total		1,021,870	37,376,416

2014 (CSO)			
NO	Plant Name	Quantity (Tons)	Cost (US \$)
1	Shelled Almonds	1,924	4,602,196
2	Almond Kernels	1,661	1,2617,000
3	Pistachio Kernels	16,311	22,517,000
4	Walnut Kernels	2,648	9,048,107
Total		20,620	44,182,107

Product Rejection Record

- Raisins and pistachio kernels several times.
Analytical results, > 18 ppb (4 ppb limit for Europe)

Transportation cost for one 20-ft container – US \$10,000

Organization or company conducting the mycotoxin analysis

- 1- Afghan Raisin Fruits & Vegetables Promotion Administration
- 2- Company (Sun power). Not Reliable

Project Objectives

Rapid Assessment (Completed) Outcome

Result & Distinguish the condemnation,

Poor storage conditions

Poor information about condemnation (Farmer and Traders)

➤ Mitigation : ?

➤ Verification : ?

Mitigation (Goal):

For reduction, what we need to do?

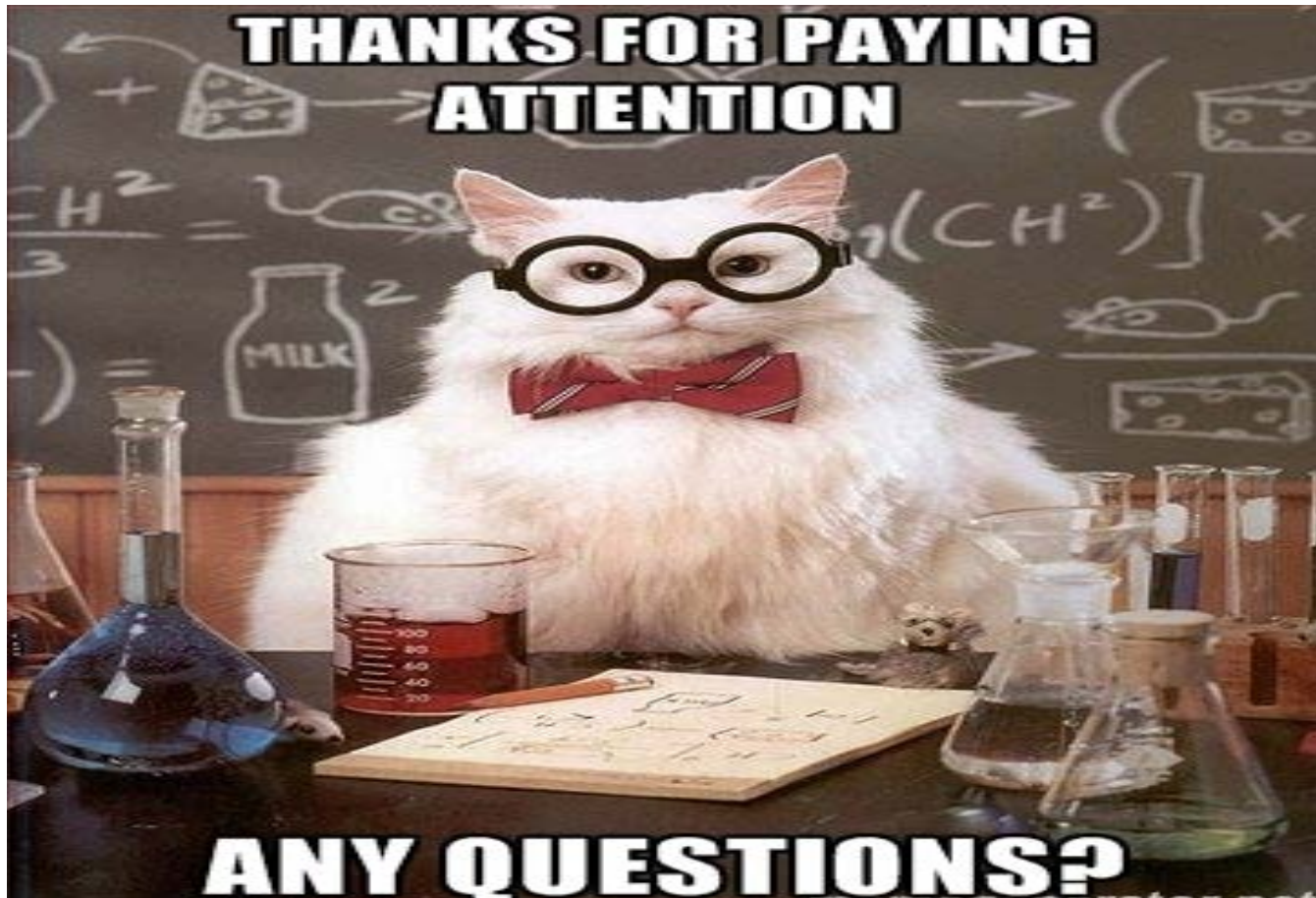
➤ Install, standard raisin storage (khishmishkhana).

➤ Air aspiration for nuts & grains.

➤ Wash product with chlorine?

➤ Increase awareness of traders and farmers.

➤ Apply for ISO. Develop and adapt support documentation



Jahed Ahadi

Director, Plant Quarantine in Kabul customs

Overall Results

John F. Leslie

Department of Plant Pathology

Kansas State University

What are Mycotoxins?

- Natural toxic metabolites produced by fungi
- Problems known at least since Ancient Greece
- Five agriculturally most important mycotoxins:
 - Aflatoxins
 - Fumonisin
 - Deoxynivalenol and other trichothecenes, *e.g.*, T-2
 - Zearalenone
 - Ochratoxin

Some also are potent carcinogens and mutagens

Several toxins are 3-5 orders of magnitude more toxic than fungicides that control them and break down more slowly

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 - Zearalenone
 - **Ochratoxin**
 - **Ergot Alkaloids**

Some also are potent carcinogens and mutagens

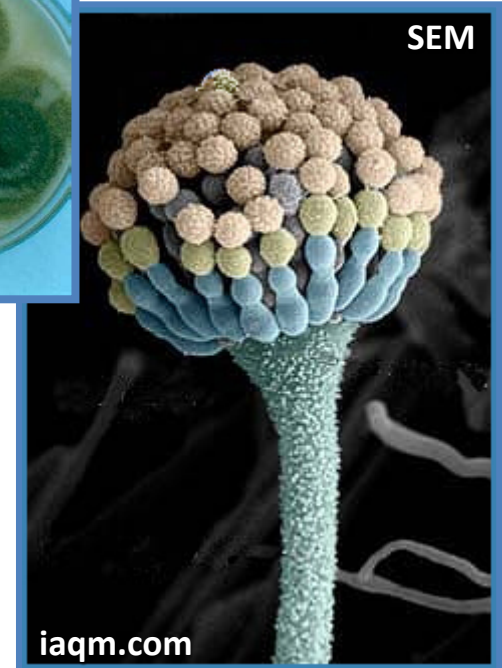
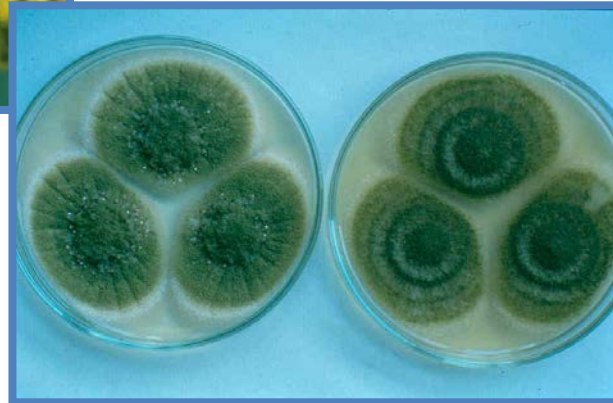
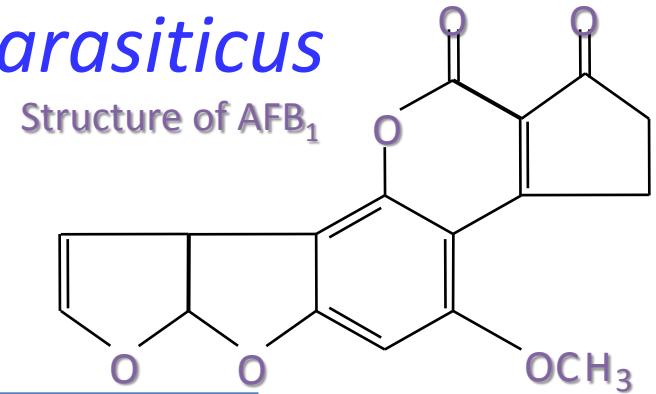
Several toxins are 3-5 orders of magnitude more toxic than fungicides that control them and break down more slowly

What Was Done?

- Afghanistan – MAIL and Deb Frey
 - Trained staff
 - Established and equipped a functional lab
 - Collected samples from across Afghanistan
 - Assayed raisins, nuts & wheat with Romer test kits
- Italy – ISPA
 - UPLC and LC-MS assays for trichothecenes in wheat
 - Mycological analyses of flour
- Austria – BOKU
 - Quadripole MS assay for 650 different metabolites
- USA – K-State and Univ. Nebraska-Lincoln
 - Assay nuts and wheat with Romer test kits
 - Test kits from Vicam and Neogen
 - Mycological analyses of flour
 - Synthesize results
 - Test reliability of test kits

Aflatoxins

Aspergillus flavus / *A. parasiticus*



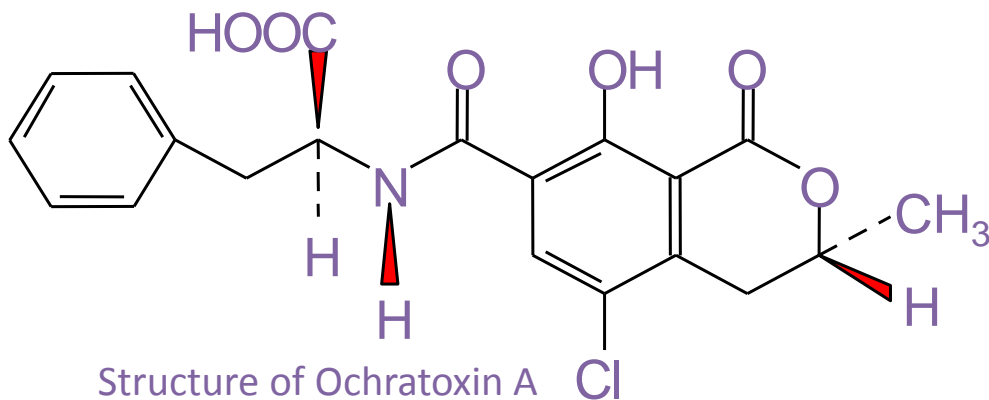
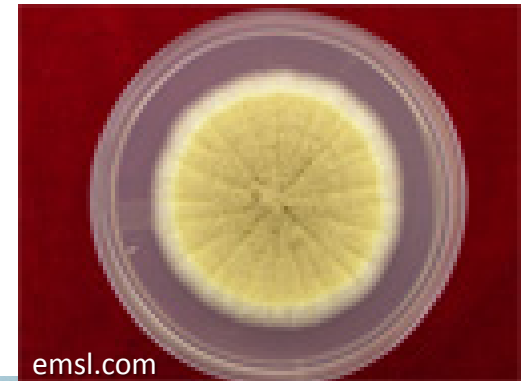
Liver failure
Liver cancer
Growth stunting
Immune deficiency or
suppression
Grains – especially maize
Peanuts
Nuts

Ochratoxins

Aspergillus ochraceus



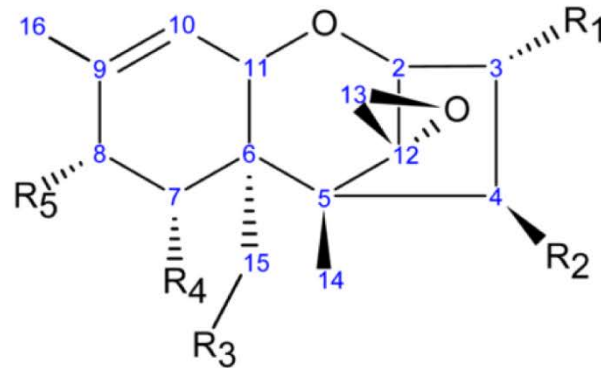
Kidney failure
Cacao
Nuts
Grapes
Coffee
Wheat



Trichothecenes

- Two classes – A & B, strains make only one type
- Both inhibit protein synthesis
- Most common in grains
- Type A – very toxic – T-2, HT-2 & DAS
 - US Select agent list
 - Purportedly used for biological warfare
- Type B – not as toxic – DON, NIV
 - More widespread, especially on wheat
- Can be taken up through skin or intestinal mucosa
- Cause vomiting, diarrhea, & immune suppression

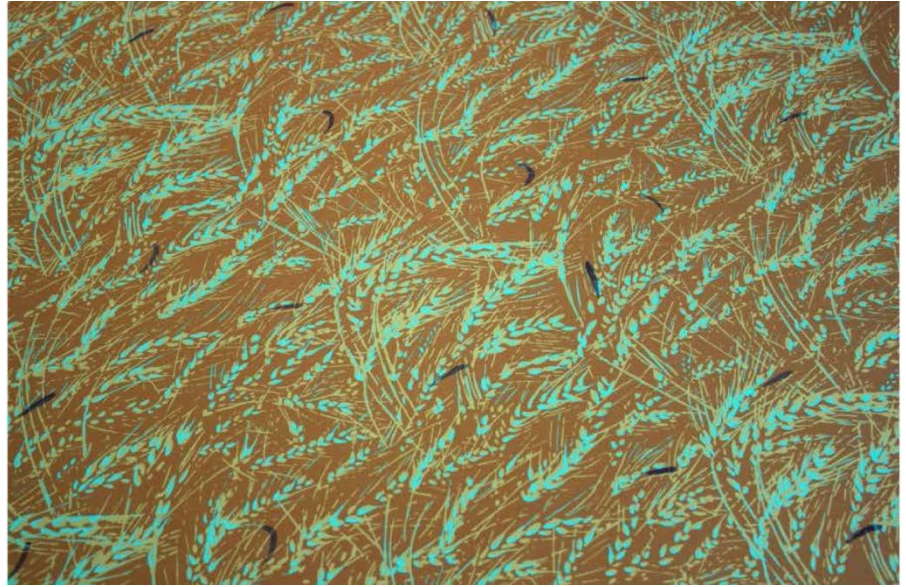
Toxin	R1	R2	R3	R4	R5
DON	-OH	-H	-OH	-OH	=O
3-ADON	-OAc	-H	-OH	-OH	=O
15-ADON	-OH	-H	-OAc	-OH	=O
NIV	-OH	-OH	-OH	-OH	=O
T-2	-OH	-OAc	-OAc	-H	-Olsoval
HT-2	-OH	-OH	-OAc	-H	-Olsoval
4,15-DAS	-OH	-OAc	-OAc	-H	-H



T-2 – Killed 1000s in Russia after WWII
DON – Becoming very widespread in US and Europe, especially where wheat and maize are grown
DON is changing the economic landscape of the US Great Plains
Fusarium is the main producer on grains, but other fungi and some plants also synthesize

Ergot Alkaloids

- Small Grains – Wheat, rye, barley & oats
- An unexpected finding by Austrian group
- Not highly regulated (animal feed only)
- In small doses – hallucinations (LSD)
- In other cases – neuropathy and gangrene
- Gnostics and ancient Greeks may have used them to help people have visions
- Controlled by sorting ergot bodies from the grain before processing



Some Ergot Epidemics

-600 – Assyria

857 – Germany

945 – France

1093 – France

1692 – USA

1926 – Russia

1929 – Ireland

1953 – France

1958 – India

1973 – Ethiopia

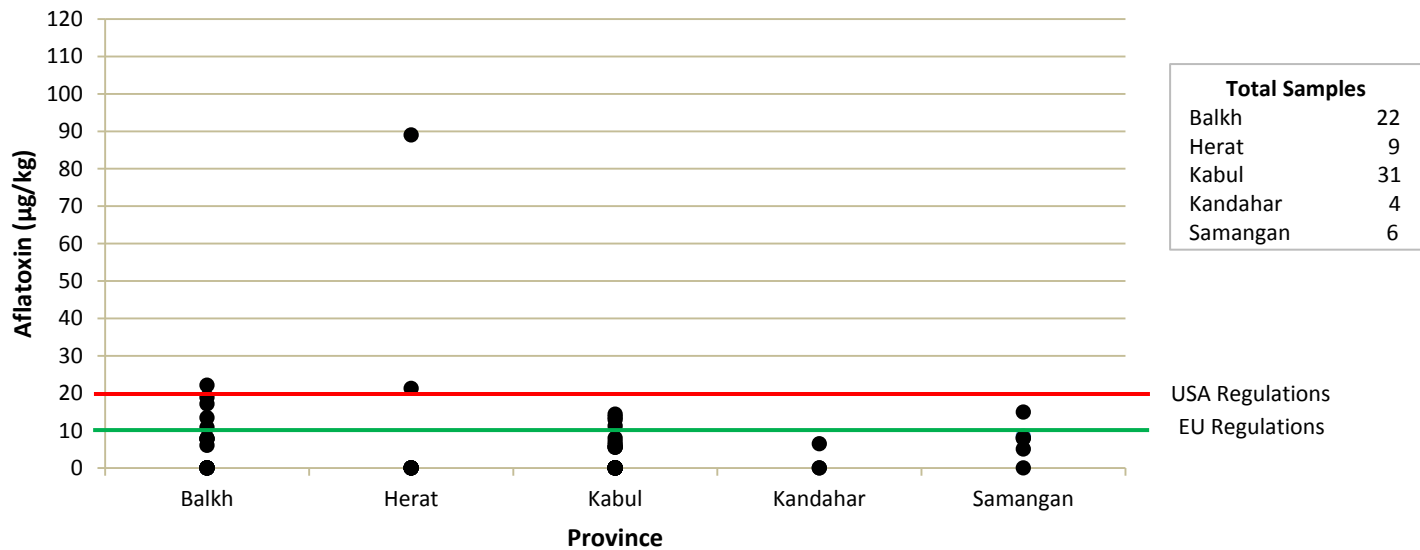
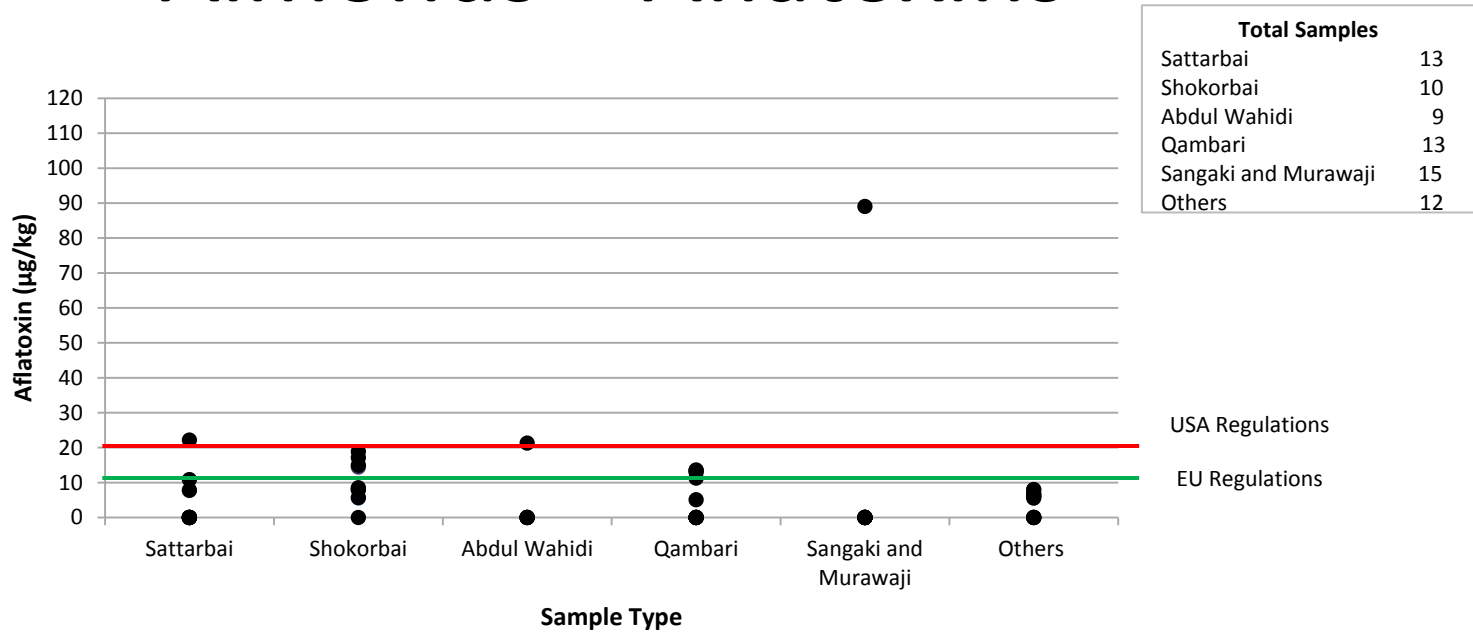
Test Kit Performance

- Romer kits for aflatoxin, ochratoxin and deoxynivalenol (DON or vomitoxin) were reliable for all tested substrates
- Vicam kits for ochratoxin and DON were reliable for wheat
- Neogen tests for aflatoxin and ochratoxin were reliable for all tested substrates
- Vicam kit for aflatoxin did not work with wheat
- Romer test for T-2 toxin was erratic
- Neogen and Romer tests for T-2/HT-2 toxins gave many (Romer) and exclusively (Neogen) false positives in wheat

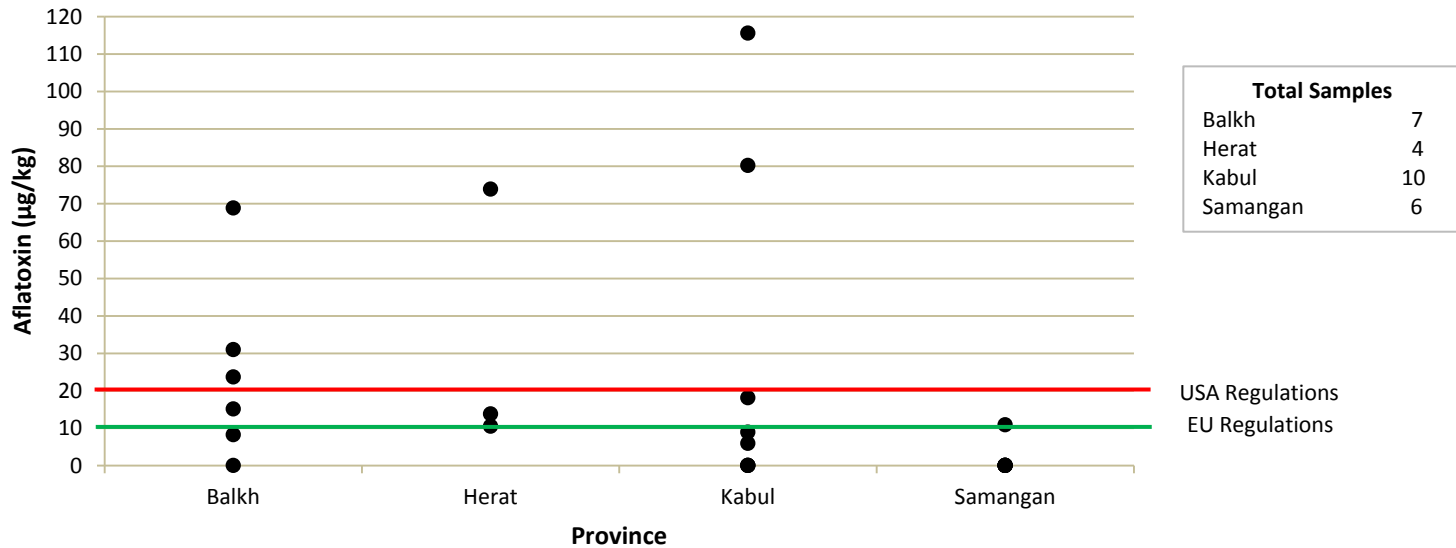
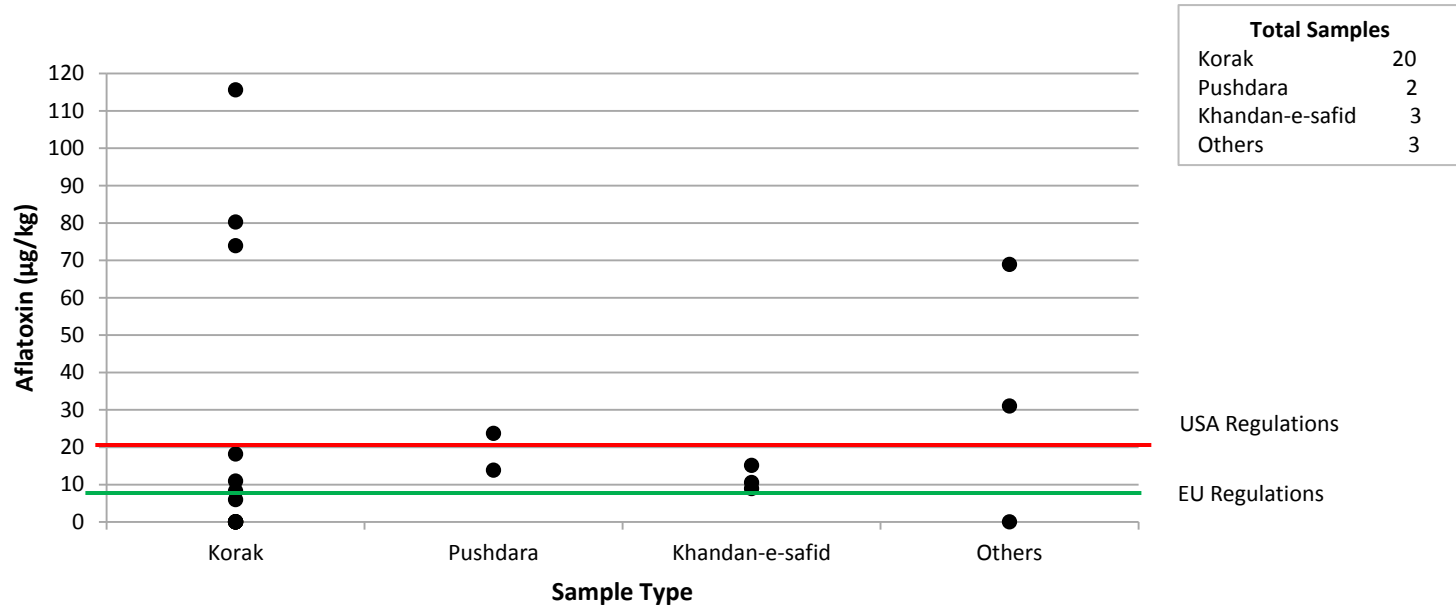
Nuts – Results

- Aflatoxin
 - Almonds – 15/81 at export limiting level
 - Pistachios – 19/40 at export limiting level
 - Walnuts – 8/25 at export limiting level
- Ochratoxin
 - Almonds – no contamination detected
 - Pistachios – 2/40 at export limiting levels
 - Walnuts – no contamination detected

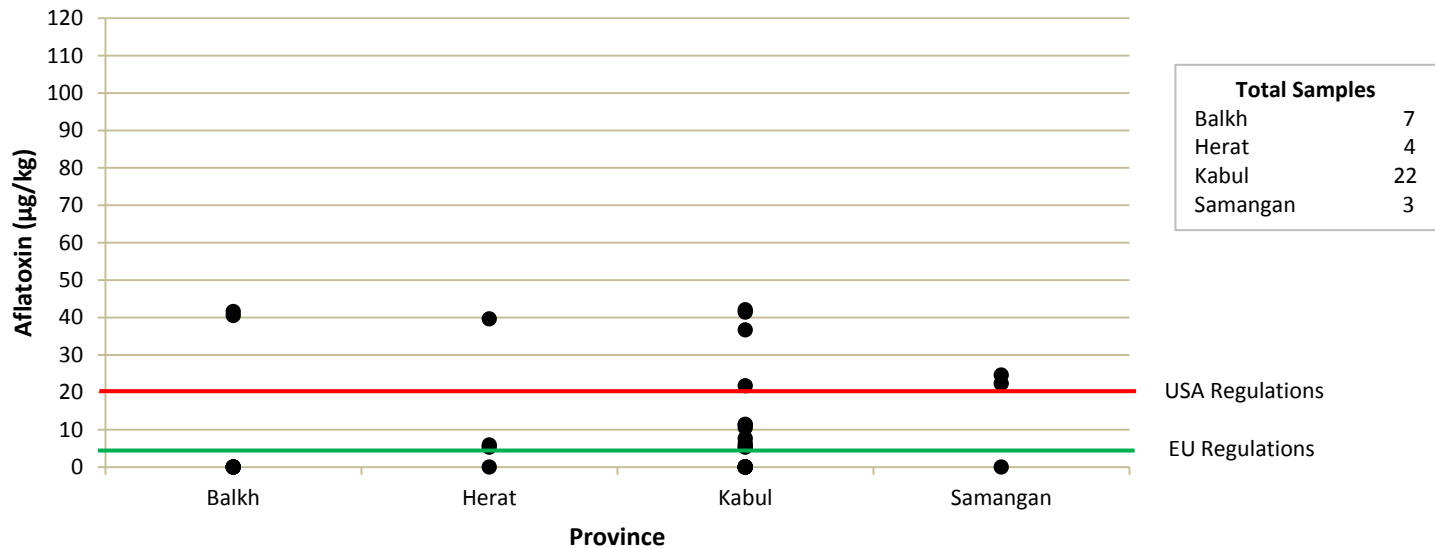
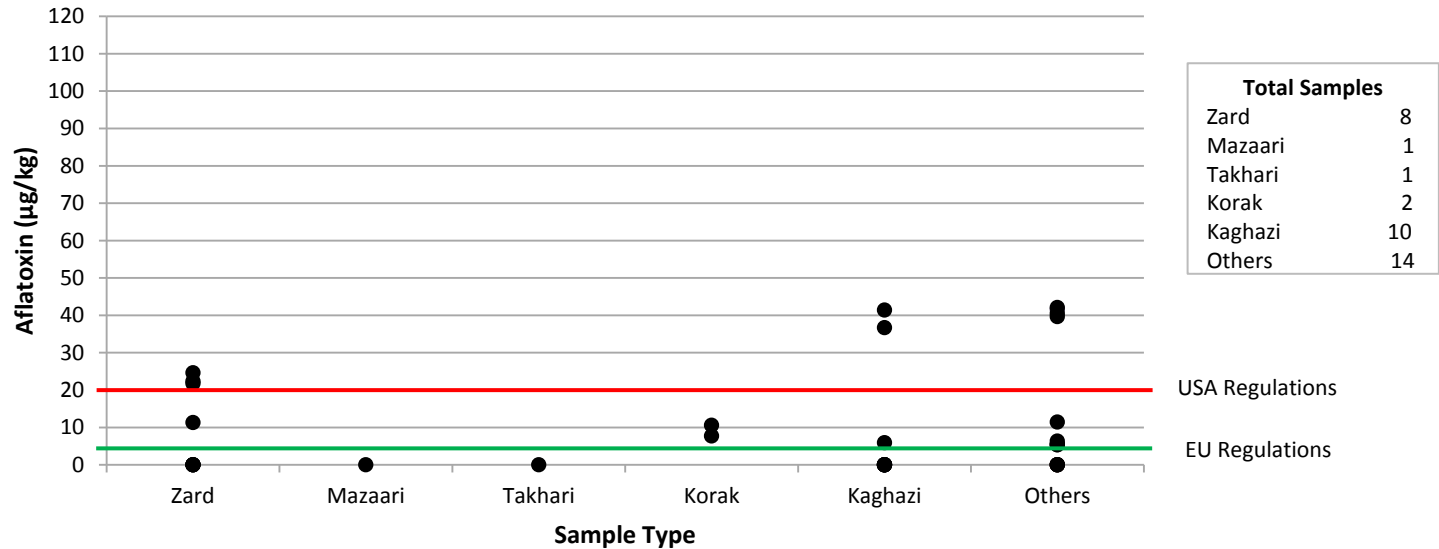
Almonds – Aflatoxins



Pistachios – Aflatoxins



Walnuts – Aflatoxins



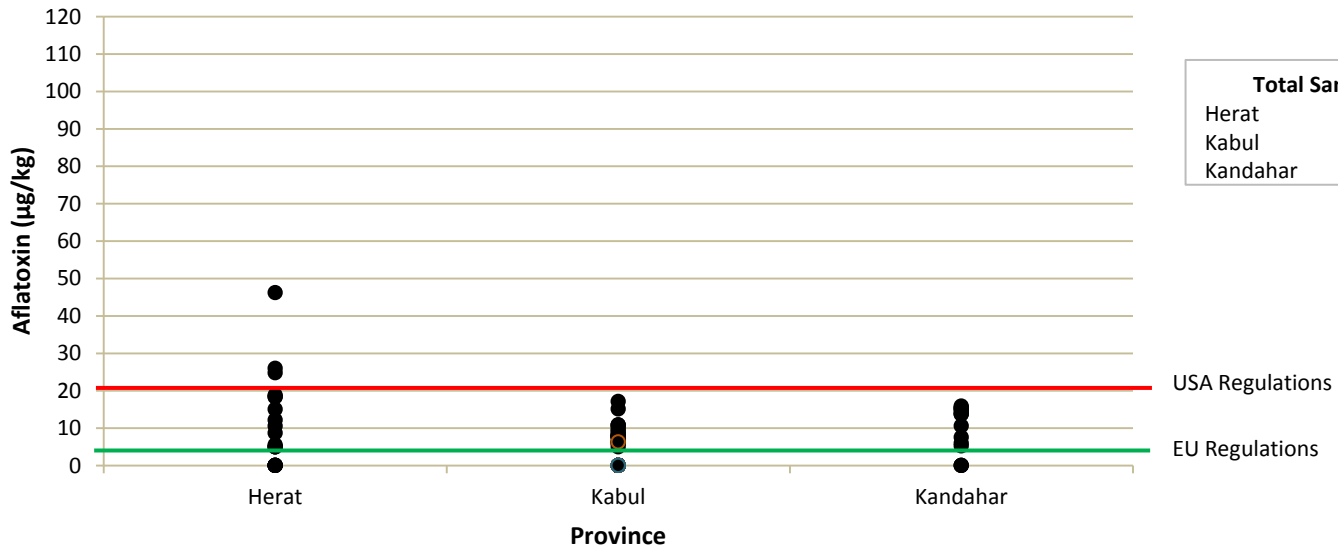
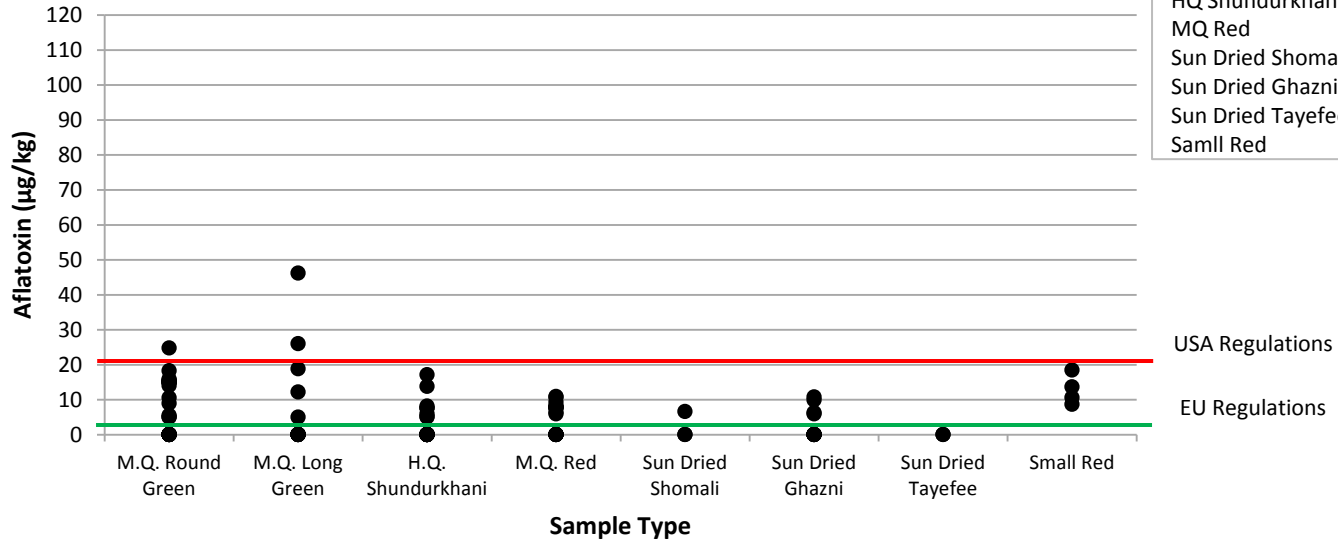
Austrian Screen – Nuts

<i>Fusarium</i>	<i>Alternaria</i>	<i>Aspergillus</i>	<i>Penicillium</i>
Butenolide	Alternariol	Cyclopiazonic Acid	Andrastin A
	Alternariol methyl ether	Aflatoxin	Andrastin B
Epiequisetin	Altersetin	Asperfuran	Agroclavine
Equisetin	Infectopyron	Kojic acid	Chanoclavin
Fusaric acid	Macrosporin	Malformin A	Epoxyagroclavin
HT-2 toxin	Tentoxin	Malformin A2	Festuclavine
T-2 toxin	Tenuazonic acid	Malformin C	Mycophenolic acid
Zearalenone			Mycophenolic acid IV
α -Zearalenol		Nigragillin	
β -Zearalenol		3-Nitropropionic acid	Penitrem A
		Ochratoxin	
		Paspalin	

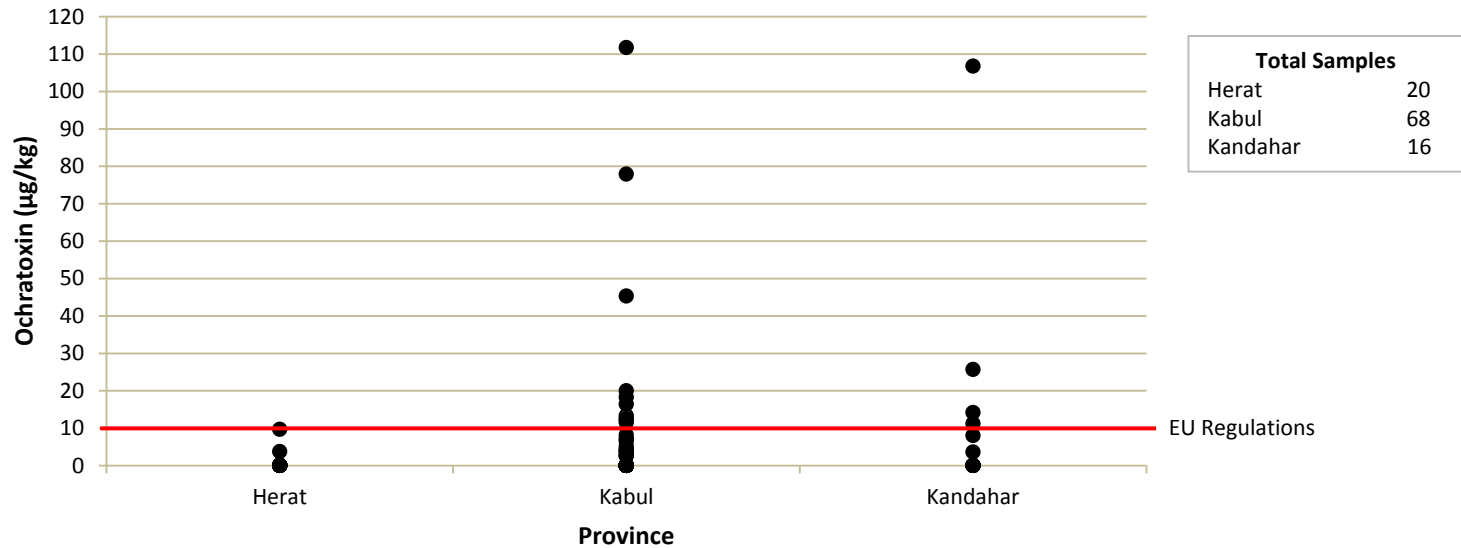
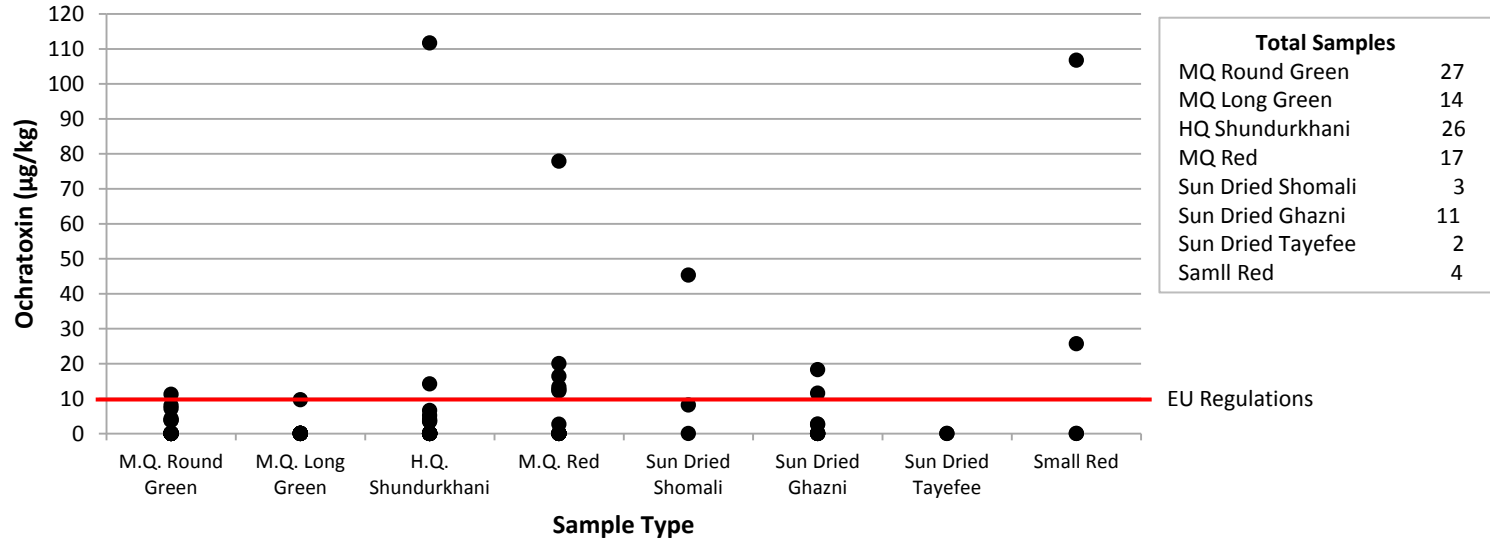
Raisins – Results

- Aflatoxins could limit exports in 43/89 samples
- Ochratoxin could limit exports in 25/80 samples
- Raisin type and drying method can be important
- Afghanistan and Austria results are discordant
- Choice of country to export to may depend on level of contamination

Raisins – Aflatoxins



Raisins – Ochratoxin



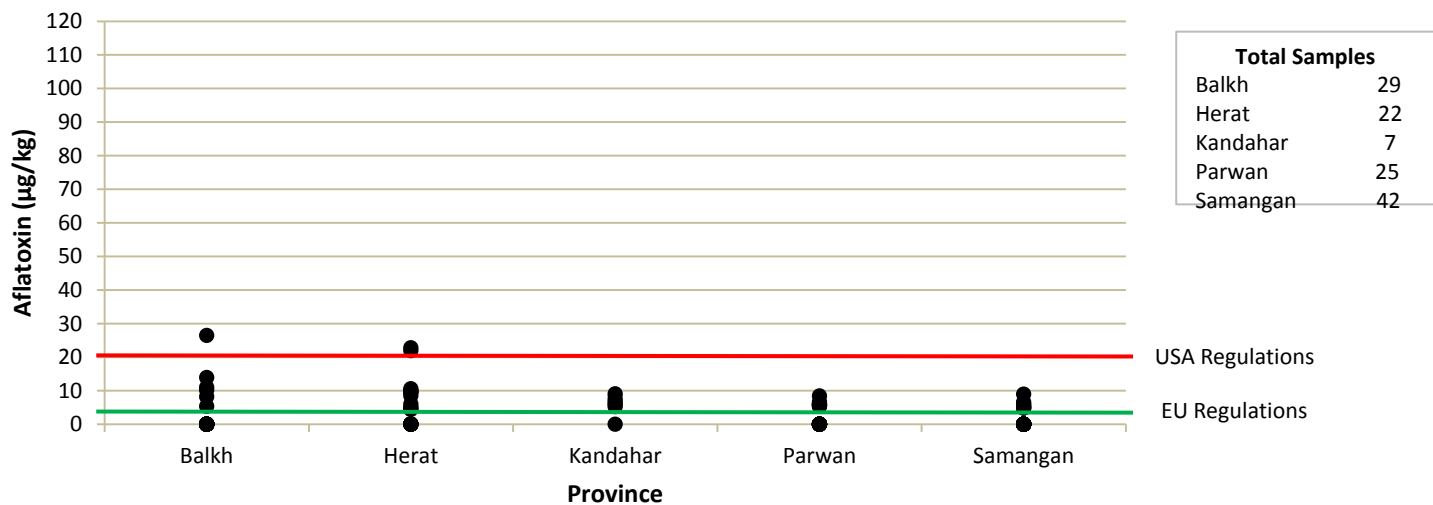
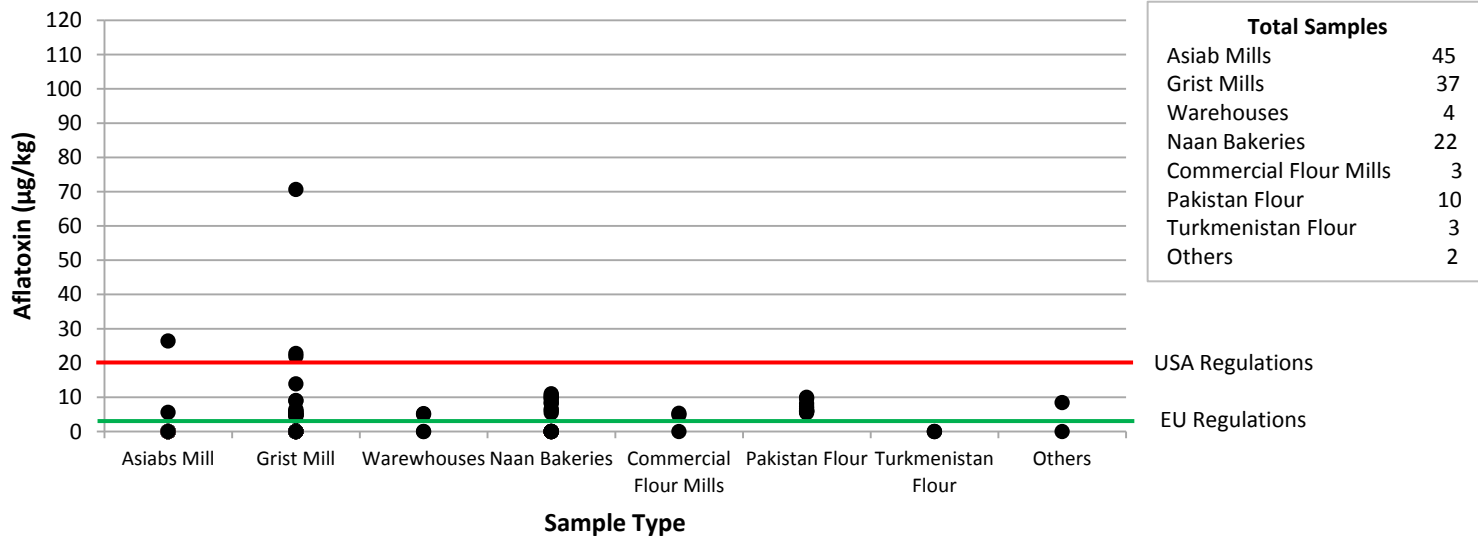
Austrian Screen – Raisins

<i>Fusarium</i>	<i>Alternaria</i>	<i>Aspergillus</i>	<i>Penicillium</i>
Fumonisin	Alternariol	Aflatoxin	Andrastin A
	Alternariol methyl ether	Aurasperon B	Andrastin B
	Altersetin	Aurasperon C	Andrastin C
	Altertoxin-I	Aurasperon G	Chanoclavin
	Macrosporin	Fonsecin	Festoclavine
	Tentoxin	Malformin A	Mycophenolic acid
	Tenuazonic acid	Malformin A2	Mycophenolic acid IV
		Malformin C	Penitrem A
		Nigragillin	Quinolactacin A
		Ochratoxin	
		Pyranonigrin	

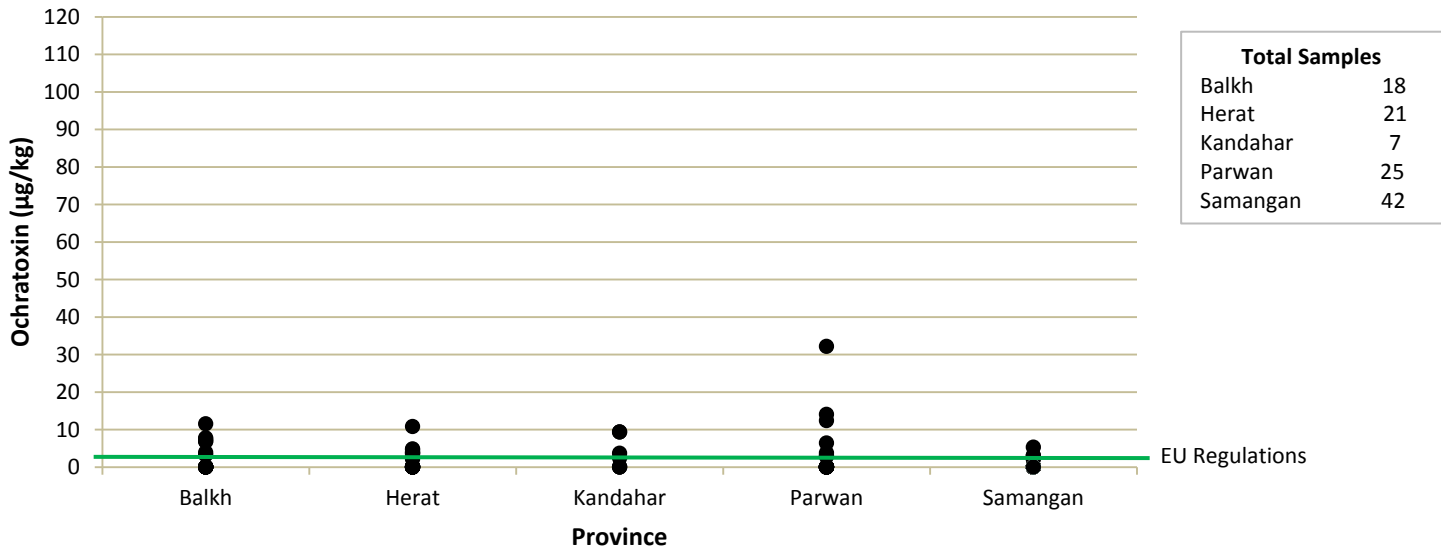
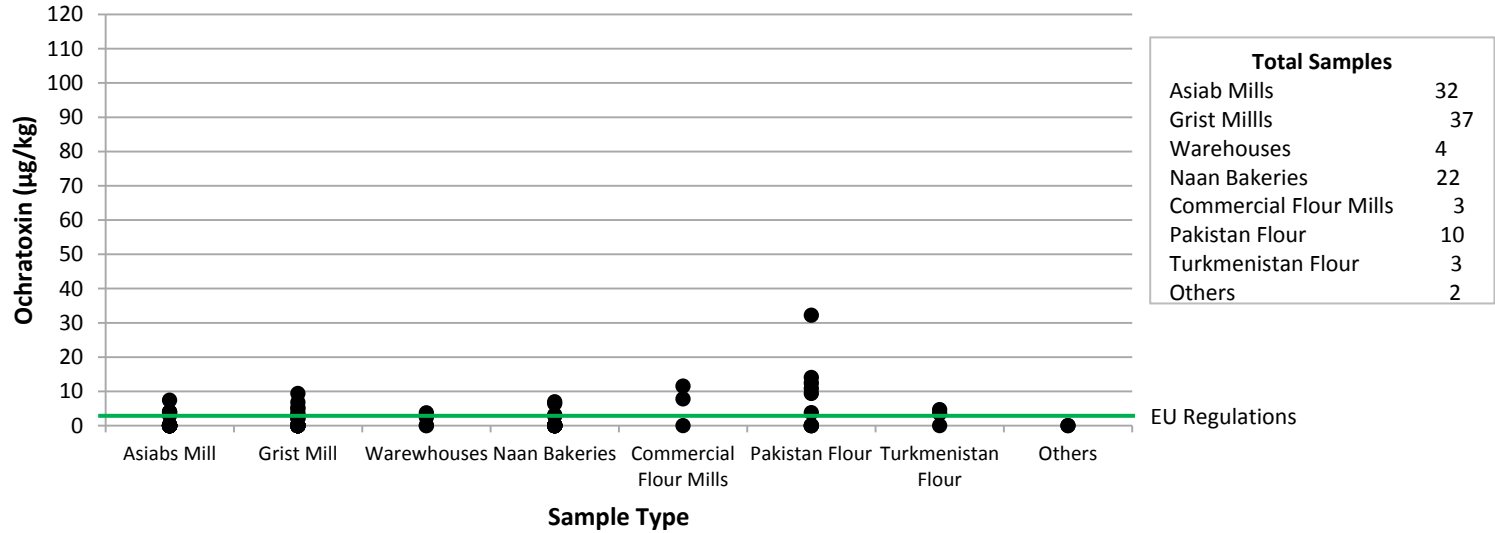
Wheat – Results

- International standards may be too high for Afghanistan safety because of the large amount of wheat consumed daily (500 g/person/day)
- Aflatoxins – detectable in 23/151 samples
 - Not a field contaminant of wheat
 - Contamination likely occurs in storage
- DON – 3/185 above international guidelines
 - Not a major problem, but exists
 - Weather and storage dependent
- Ochratoxin – detectable in 36/181
 - Common problem in northern Europe
 - Needs attention
 - May carry over to meat
- T-2 and HT-2 – Not reliably detected
- Ergot – detectable 51/151
 - High incidence
 - Easily remedied by cleaning grain

Wheat - Aflatoxin



Wheat – Ochratoxin



Austrian Screen – Wheat

<i>Fusarium</i>	<i>Alternaria</i>	<i>Aspergillus</i>	<i>Penicillium</i>	<i>Claviceps</i>
Beauvericin	Alternariol	Aflatoxin	Agroclavine	Ergocristine
Enniatin A	Alternariol methyl ether	Averantin	Chanoclavine	Ergocristinine
Enniatin A ₁	Altersetin	Averufin	Citrinin	Ergometrine
Enniatin B	Altersolanol	Cycloaspeptide A	Chrysogine	Ergometrinine
Enniatin B ₁	Altertoxin-I	Kojic acid	Elymoclavine	Ergosin
Epiequisetin	Macrosporin	Methoxysterigm atocystin	Mycophenolic acid	Ergosinin
Equisetin	Tentoxin	3-Nitropropionic acid	Questiomycin A	Ergotamine
HT-2 toxin	Tenuazonic acid	Norsolorinic acid	Quinolactacin A	Ergotaminine
T-2 toxin		Ochratoxin	Secalonic acid D	
Zearalenone		Sterigmatocystin		

Conclusions

- The test kit used matters
- Sampling procedures are critical
- Pre- and Post-harvest processes both matter
- Nuts and aflatoxins – Pistachios > Walnuts > Almonds
- Raisins – Aflatoxins > Ochratoxin
 - Type of raisin and drying method important
- Wheat
 - Need to evaluate safety levels for Afghan diets
 - Aflatoxin is a storage issue
 - *Fusarium* toxins (T-2, HT-2, DON & Zearalenone) are minimal
 - Citrinin + ochratoxin could enhance kidney problems
 - Ergot – high frequency, but relatively easy to fix



Special thanks to all of our collaborators in Afghanistan, Austria, Nebraska, Kansas (and my wife!).

Questions?

“Where waters are
murky, crocodiles lurk!”

- Old African saying



Sampling Collection protocols

By
Asadullah Ansari

Plant Protection and Quarantine Directorate
Laboratory .

MAIL
3/14/2016

(Definition)

A sample is the specific amount of a commodity which represents a complete lot.



Samples Collection Areas

Herat, Kandahar, Balkh, Samangan, Kabul, Kapisa, and Parwan.



Standard method for sample collection

- ▶ Nuts without shell 1Kg.
- ▶ Nuts with shell 2Kg.
- ▶ Dried fruit 1 Kg
- ▶ Flour and wheat one 1 Kg.



10.01.2015 10:03

Weighing



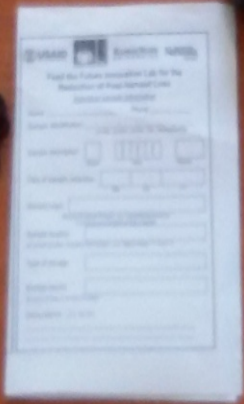
Objective

1. Sample collection for identifying mycotoxin.
2. Storage observation .
3. Storage duration.
4. Storage sanitation.



Pre-Sampling Activities

1. Identify sampling area.
2. Equipments preparation for sampling: e.g. sample collection bags, gloves, mask, transferring bag, UV-light, sample ID form, pen, camera, wet tissue paper, beaker, sample stick, GPS.



Lot sampling

- Lot is a total of a product which is a representative sample randomly, it means from the top, below and center, then collect those in a sample bag.
- If numbers of bags are 1 – 20 in a storage the sample should be collected from all the bags.
 - if numbers of bags are 21 – 1000 bags, samples should be collected 6% (20 samples)
 - if it is more than 1000, samples should be collected 3% (60 Samples) and the sampling must be collected randomly.



09.08.2015 10:14



Mill Sampling



Open Area Sampling



observations



Physical observations

Smut, fusarium wilt, kernel bunt of wheat, black point, dark spots on raisin, shrunken grain, stone and grit in walnut, almond and etc.



Sample Collection Form

USAID **KANSAS STATE UNIVERSITY** **UNIVERSITY OF NEBRASKA**
Lincoln

Feed the Future Innovation Lab for the Reduction of Post-Harvest Loss

Individual sample information

Name: _____ Phone: _____

Sample identification: _____
commodity - province - sampler - date - sequential number

Sample description: Wheat Nuts Raisins

Date of sample collection: _____
MM DD YY

Sample origin: _____
(Source of sample if known; e.g.: harvested/produced at X province or purchased at Kabul market)

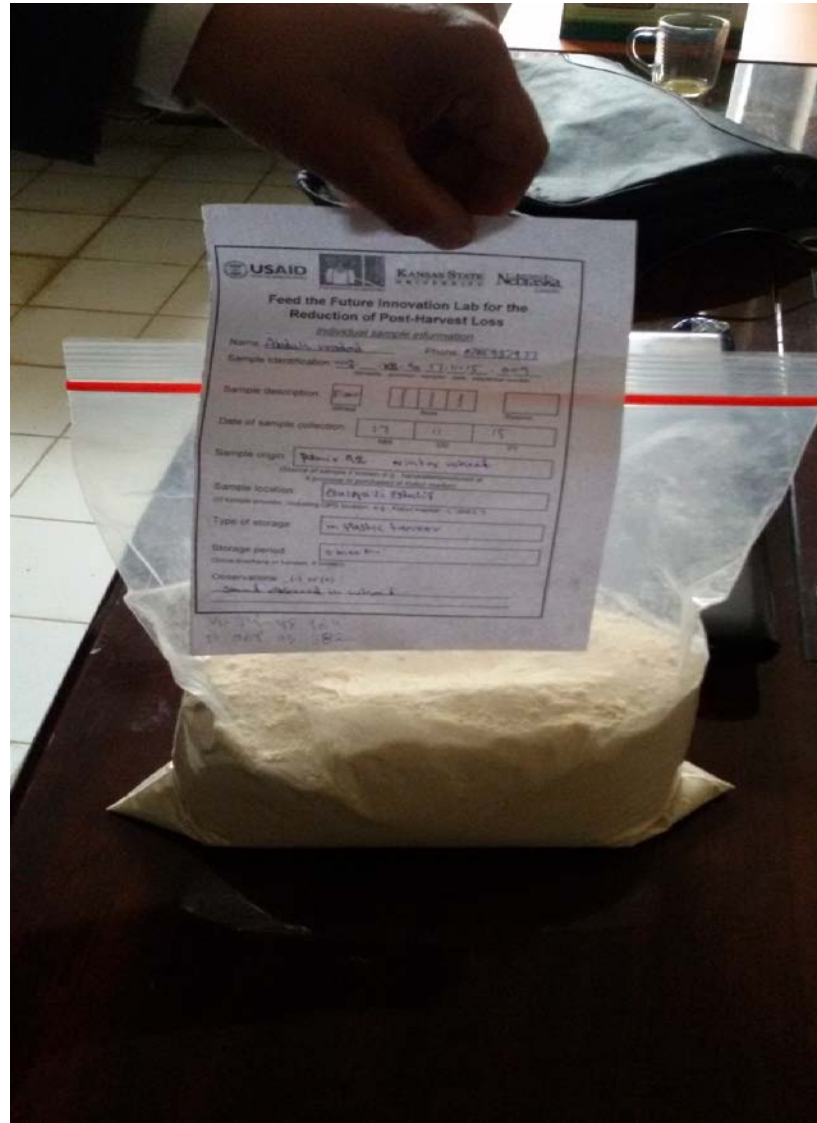
Sample location: _____
(of sample provider, including GPS location, e.g.: Kabul market - L¹ and L²)

Type of storage: _____

Storage period: _____
(Since purchase or harvest, if known)

Observations: _ (-) or (+)

Completed Sample Collection Form



Collected sample must be transferred in a bag.



Thanks for your attention

Standard Operating Procedures for Collection, Handling and Storage of Data

1.1 Sample Information Form

1.2 Sampling Control Log - Field Form

2. Sample Processing

2.1 Sampling Control Log - Lab

2.2 ELISA (Enzyme-linked immunoabsorbent assay) Control Log

2.3 Data Worksheets

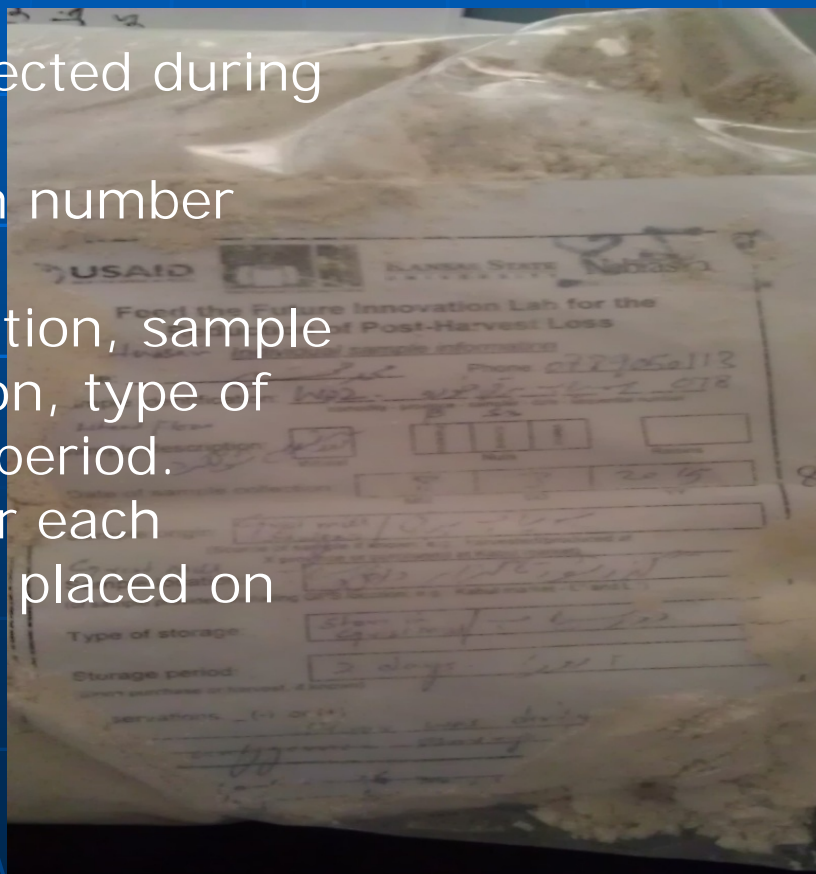
3. Data Storage and Protection

The results obtained in the analysis of a particular mycotoxin in each sample must be properly transcribe onto the **Elisa Control Log Form (ECLF)**.

Individual Sample Information Form (ISIF)

The information collected during sampling include sample identification number, sample description, date of sample collection, sample origin, sample location, type of storage and storage period.

must be filled out for each sample collected and placed on the sample



USAID FROM THE AMERICAN PEOPLE

KANSAS STATE UNIVERSITY

UNIVERSITY OF NEBRASKA LINCOLN

Post-Harvest Loss Reduction

Feed the Future Innovation Lab for the Reduction of Post-Harvest Loss

Individual sample information

Name: _____ Phone: _____

Sample identification: _____
commodity - province - sampler - date - sequential number

Sample description: Wheat Nuts Raisins

Date of sample collection: _____
MM DD YY

Sample origin: _____
(Source of sample if known, e.g.: harvested/produced at X province or purchased at Kabul market)

Sample location: _____
(of sample provider, including GPS location, e.g.: Kabul market - 'L' and 'L')

Type of storage: _____

- 2. Sample Processing
- 2.1 Sampling Control Log - Lab(SCLLF).

When the SCLLF form has been received, the form should be date stamped and reviewed for any missing data, incomplete fields or data outside normal ranges.

- field identification
- laboratory identification



KANSAS STATE UNIVERSITY

UNIVERSITY OF Nebraska Lincoln

Feed the Future Innovation Lab for the Reduction of Post-Harvest Loss

Sampling control: Laboratory

Sample ID		Responsible for sampling	Sample location (provider, GPS)	Sample receiving date (MM/DD/YY)	Observations
Field ID <u>wal-B-9-8-5-2015-093</u> commodity - province - sampler - date - sequential number		Jahed Rustami	lat: 36.11053877 long: 119.4700	8-10-2015	
Lab ID <u>wal-B-9-8-5-2015-020</u> commodity - province - sampler - date - sequential number - lab number					
Date of sample collection (MM/DD/YY)	Type of storage	Sample description	Sample origin	Storage period	Responsible for receiving
8-5-2015	inside well	Flour, Bulk pomic	Bulk pomic	2 days	PPD Lab

A4B4

Sample ID		Responsible for sampling	Sample location (provider, GPS)	Sample receiving date (MM/DD/YY)	Observations
Field ID <u>wal-B-52-8-4-2015-064</u> commodity - province - sampler - date - sequential number		bari Zalcia	lat: 34.3217 6600 long: 69.7731 4999	8.8-2015	no fungus observed
Lab ID <u>wal-B-52-8-4-2015-064-022</u> commodity - province - sampler - date - sequential number - lab number					
Date of sample collection (MM/DD/YY)	Type of storage	Sample description	Sample origin	Storage period	Responsible for receiving
8-4-2015	inside shop Okajju	okajju of	okajju of	2 days ago	PPD Lab

Elisa Control Log form

The results obtained in the analysis of a particular mycotoxin in each sample must be properly transcribe onto the Elisa Control Log Form(ECLF).

The information recorded in this form include laboratory sample identification number, extraction number, sample type, mycotoxin, pH, OD value, among others.

14 2015

09:57:20

Elisa Control Log - Agravision

Sample ID	Rep A or B	Sample type	Operator	Mycotoxin	pH		OD*	Additional dilution	Final OD
					Before	After			
A04-KBL-S1-10-4-15		Almond	Jahed	Afla					
339-007	A1		Rushani		7.82		1.292		
A04-KBL-S1-10-4-15			Nasey						
339-007	B1				7.55		1.407		
A07-KBL-S1-10-3-15									
339-006	A2				6.87		1.371		
A07-KBL-S1-10-3-15									
339-006	B2				6.92		1.124		
A04-KBL-S1-10-3-15									
330-008	A3				7.30		1.283		
A04-KBL-S1-10-3-15									
330-008	B3				7.03		1.253		
A07-KBL-S1-10-3-15									
315-008	A4				7.41		1.297		
A07-KBL-S1-10-3-15									
315-008	B4				7.31		1.217		
A06-KRL-S1-10-4-15									
383-011	A5				6.77		1.330		

Printed Sheet

Read the strips with the **StatFax 4700** using a 450 nm filter with a differential filter of 630nm. Record OD readings for each microwell. Refers to the Quick Start Guide for StatFax 4700 if needed. Use the **Romer® Log/Logit AQ Afla_4-40 ppb** spreadsheet to interpret the results

OD Result

Strip	Carrier	Position	ADs	ppb	Interp
Strip: 1	Carrier	Position: 1			
Running			1.429		0.0
A	S1		1.284		4.0
B	S2		0.922		10.0
C	S3		0.466		20.0
D	S4		0.116		40.0
E	S5		1.562		0.0
F	1		1.520		0.0
G	2		1.297		1.2
H	3				
r=-0.9682 y=1.3377					
m=-0.0334					
Strip: 2	Carrier	Position: 2			
A	4		-1.190		4.4
B	5		-0.002		40.1
C	6		-0.002		40.1
D	7		-0.002		40.1
E	8		-0.002		40.1
F	9		-0.001		40.1
G	10		-0.002		40.1
H	11		-0.002		40.1
Strip: 3	Carrier	Position: 3			
A	12		0.000		40.1
B	13		0.000		40.1
C	14		0.000		40.1
D	15		0.000		40.1
E	16		0.000		40.1
F	17		0.000		40.1
G	18		0.000		40.1
H	19		0.000		40.1

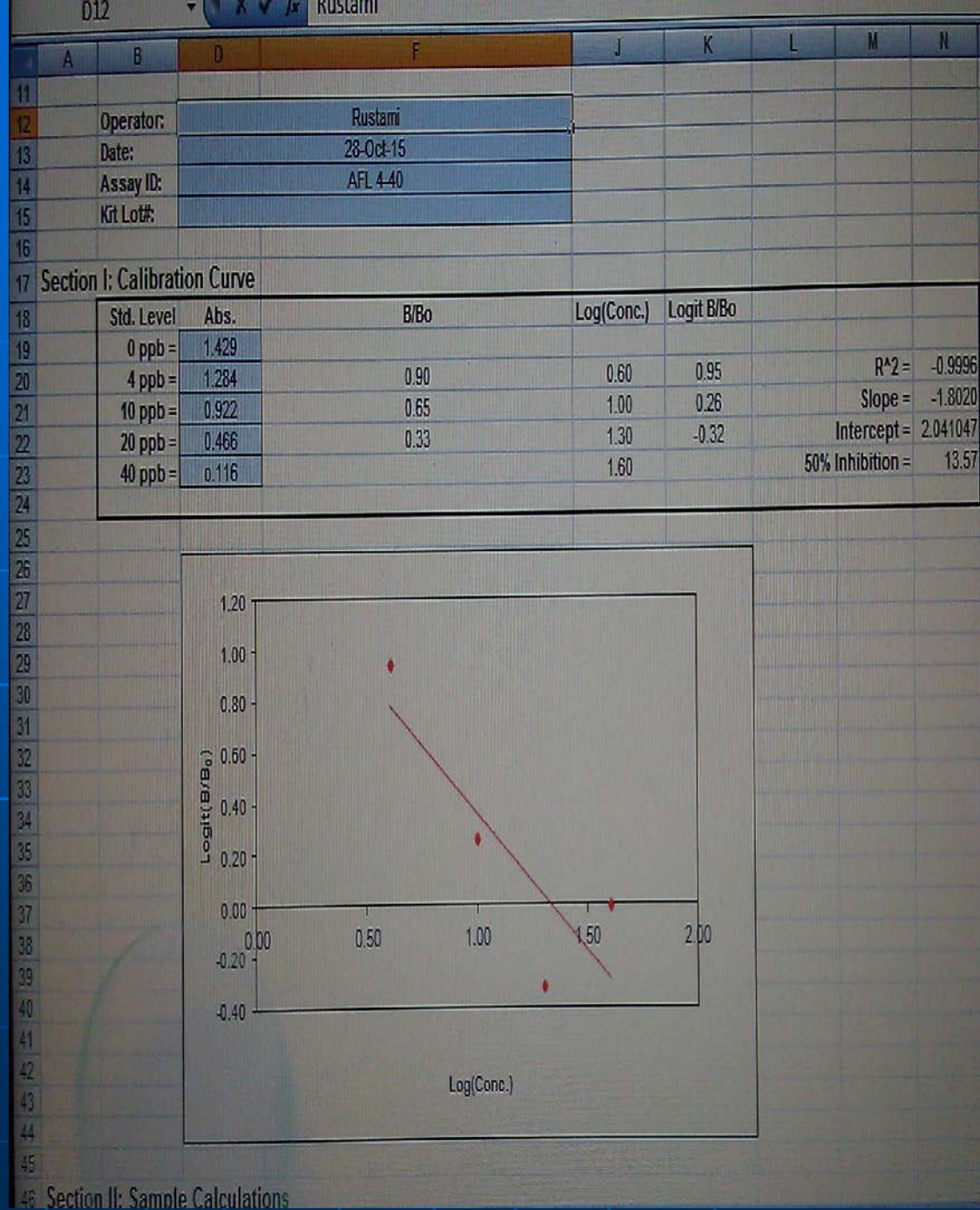


Optical Density (OD)

Result , enter the OD result spreadsheet to the System
System will Analyzing the enter Date
The Final result will appear in screen

Data Worksheets

Use the mycotoxin-specific Romer Log/Logit spreadsheet to interpret the results obtained in the analysis of a particular mycotoxin analysis are responsible for properly transcribing the OD values obtained from each standard into the Section I of the spreadsheet to build the calibration curve,



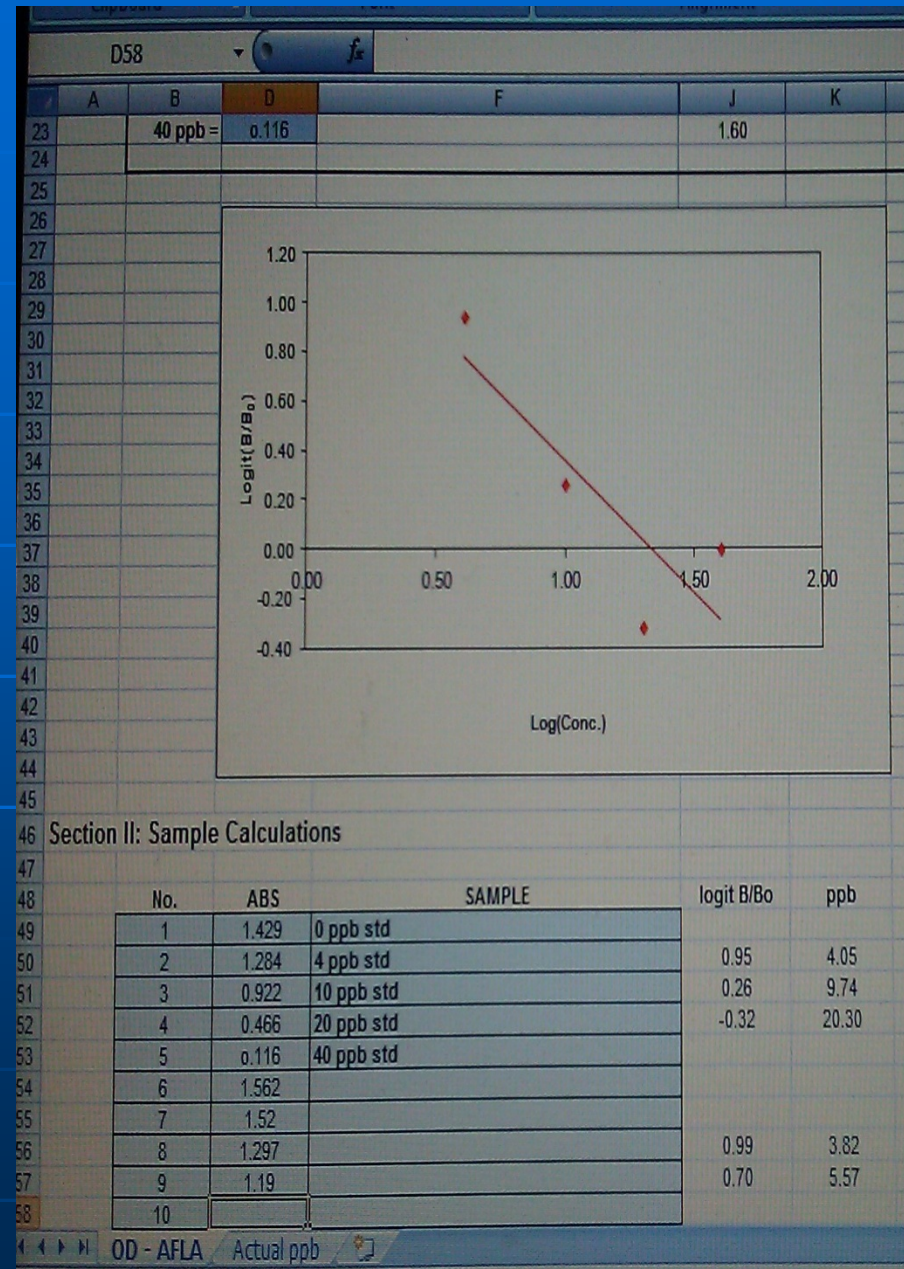
Section II

type the sample identification and the OD values obtained from each sample into the Section II of the spreadsheet, and to transcribe the pH value, preparation method and dilution for each sample into the Section III to calculate the actual mycotoxin concentration.

3. Data Storage and Protection

All the information collected in the data collection forms must be captured into an electronic record. Electronic records must be kept in a secure drive

Backups should be stored securely in a different location from the original data



Section III

and to transcribe the pH value, preparation method and dilution for each sample into the Section III to calculate the actual mycotoxin concentration

Kit Lot #: 0

Section III: Sample Calculations - Final ppb

No.	SAMPLE ID	ppb
1	0 ppb std	
2	4 ppb std	
3	10 ppb std	
4	20 ppb std	
5	40 ppb std	

No.	SAMPLE ID	pH	ppb	Actual ppb	Slurry (S) or Dry Milling (DM)?	ppb after Dilution Factor	Extra Dilution	Extra Dilution Factor	Final ppb	Average ppb	Sldev
6	0				Slurry	#VALUE!			#VALUE!	#VALUE!	#VALUE!
7	0				Slurry	#VALUE!			#VALUE!	#VALUE!	#VALUE!
8	0				Slurry	#VALUE!			#VALUE!	#VALUE!	#VALUE!
9	0				Slurry	#VALUE!			#VALUE!	#VALUE!	#VALUE!
10	0									#DIV/0!	#DIV/0!
11	0									#DIV/0!	#DIV/0!
12	0									#DIV/0!	#DIV/0!
13	0									#DIV/0!	#DIV/0!
14	0									#DIV/0!	#DIV/0!
15	0									#DIV/0!	#DIV/0!
16	0									#DIV/0!	#DIV/0!
17	0									#DIV/0!	#DIV/0!
18	0									#DIV/0!	#DIV/0!
19	0									#DIV/0!	#DIV/0!
20	0									#DIV/0!	#DIV/0!
21	0									#DIV/0!	#DIV/0!
22	0									#DIV/0!	#DIV/0!
23	0									#DIV/0!	#DIV/0!
24	0									#DIV/0!	#DIV/0!
25	0									#DIV/0!	#DIV/0!
26	0									#DIV/0!	#DIV/0!
27	0									#DIV/0!	#DIV/0!
28	0									#DIV/0!	#DIV/0!

pH
Type the sample pH

00 - ARLA Actual ppb

Thanks



Thanks





Mycotoxin Analysis: Effect of each step on data reliability

Luis Sabillón
University of Nebraska – Lincoln





Overview

There are sources of variability at each of the 3 steps of mycotoxin testing (i.e., sampling, sample preparation and analysis).

Sampling variability is the largest source of error in determining mycotoxin levels.

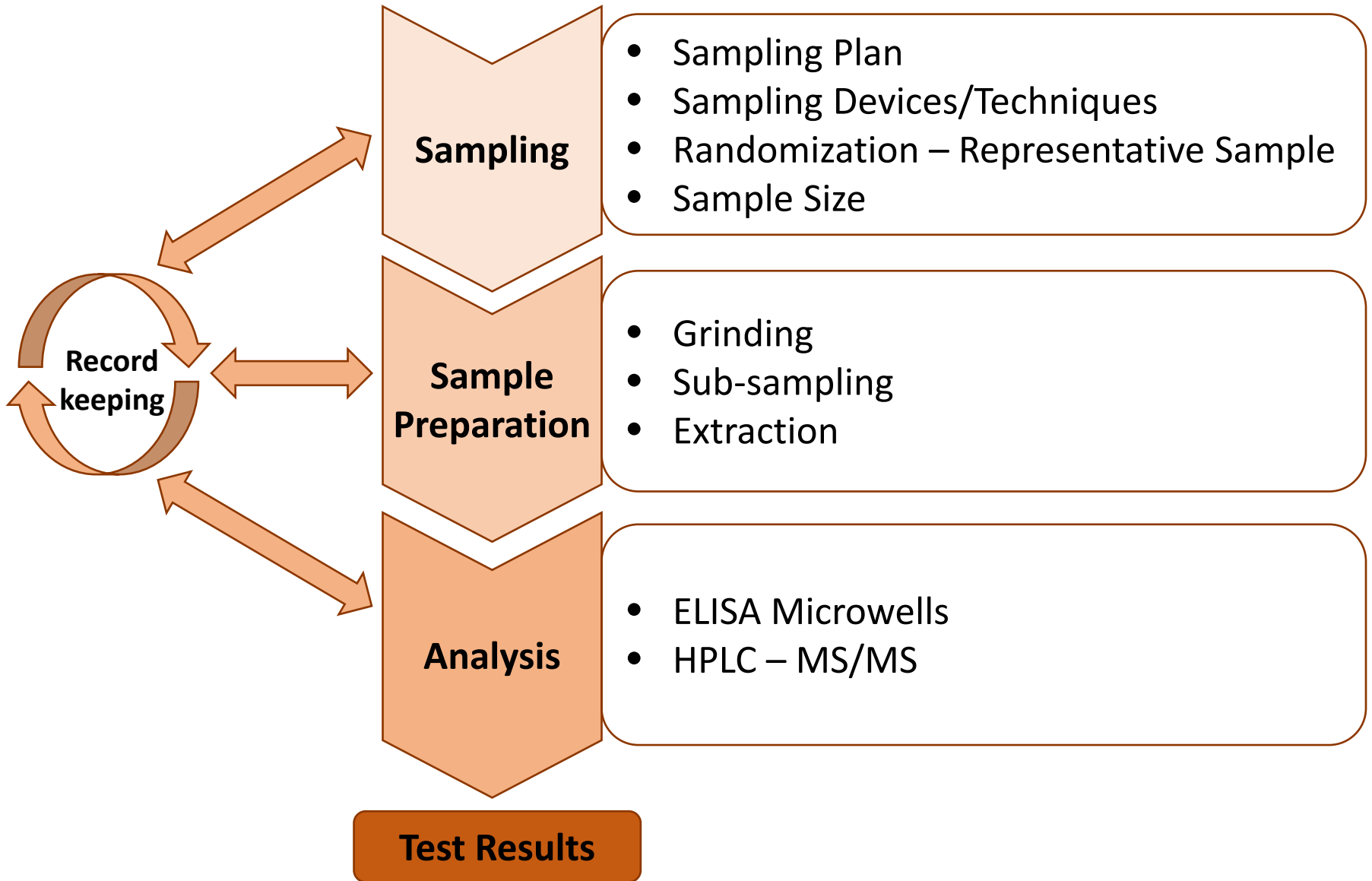
The high sampling error is due to two main factors:

- Low concentration of mycotoxins in a given commodity
- Uneven distribution in the lot

False negatives vs False positives

A crucial aspect of mycotoxin analysis is its replicability
High replicability = low variability

Mycotoxin Analysis – Main Steps



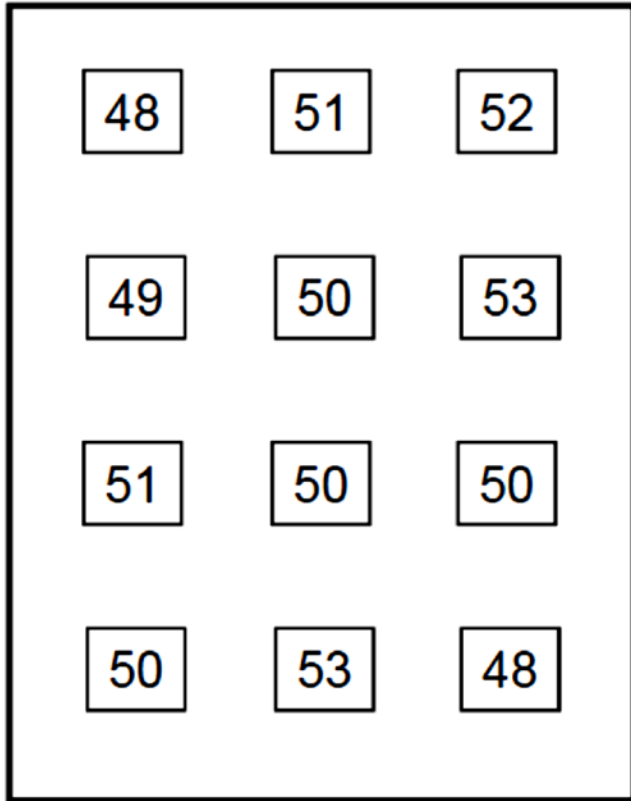
Sampling

- Major source of error and variation
- Mycotoxins are not evenly distributed in a lot
- Not every kernel or nut is contaminated
- A few kernels can contaminate large lots
- **“Cherry-picking”**

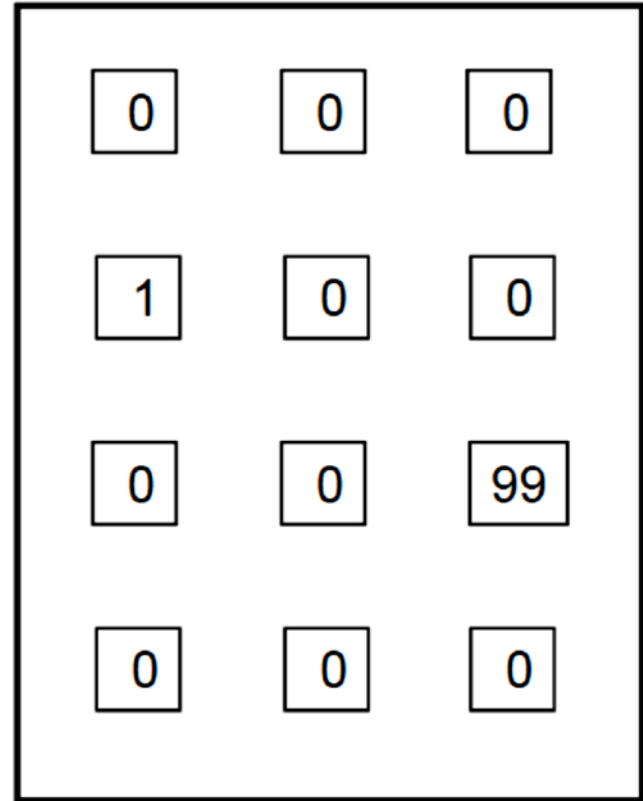




Sampling



Typical Protein Distribution

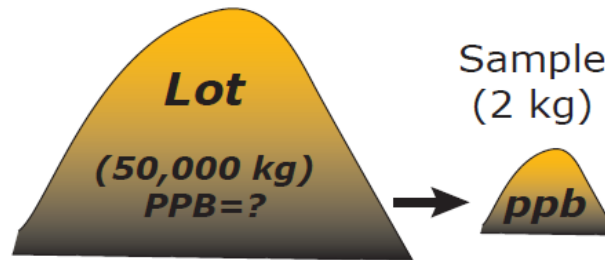


Typical Mycotoxin Distribution

Sampling

For a sample to be considered representative, it must be:

- ✓ Obtained with appropriate equipment and procedures designed to collect sample from all areas of the lot
- ✓ Of appropriate size
- ✓ Adequately identified and labeled
- ✓ Handled in such a way as to maintain its representativeness



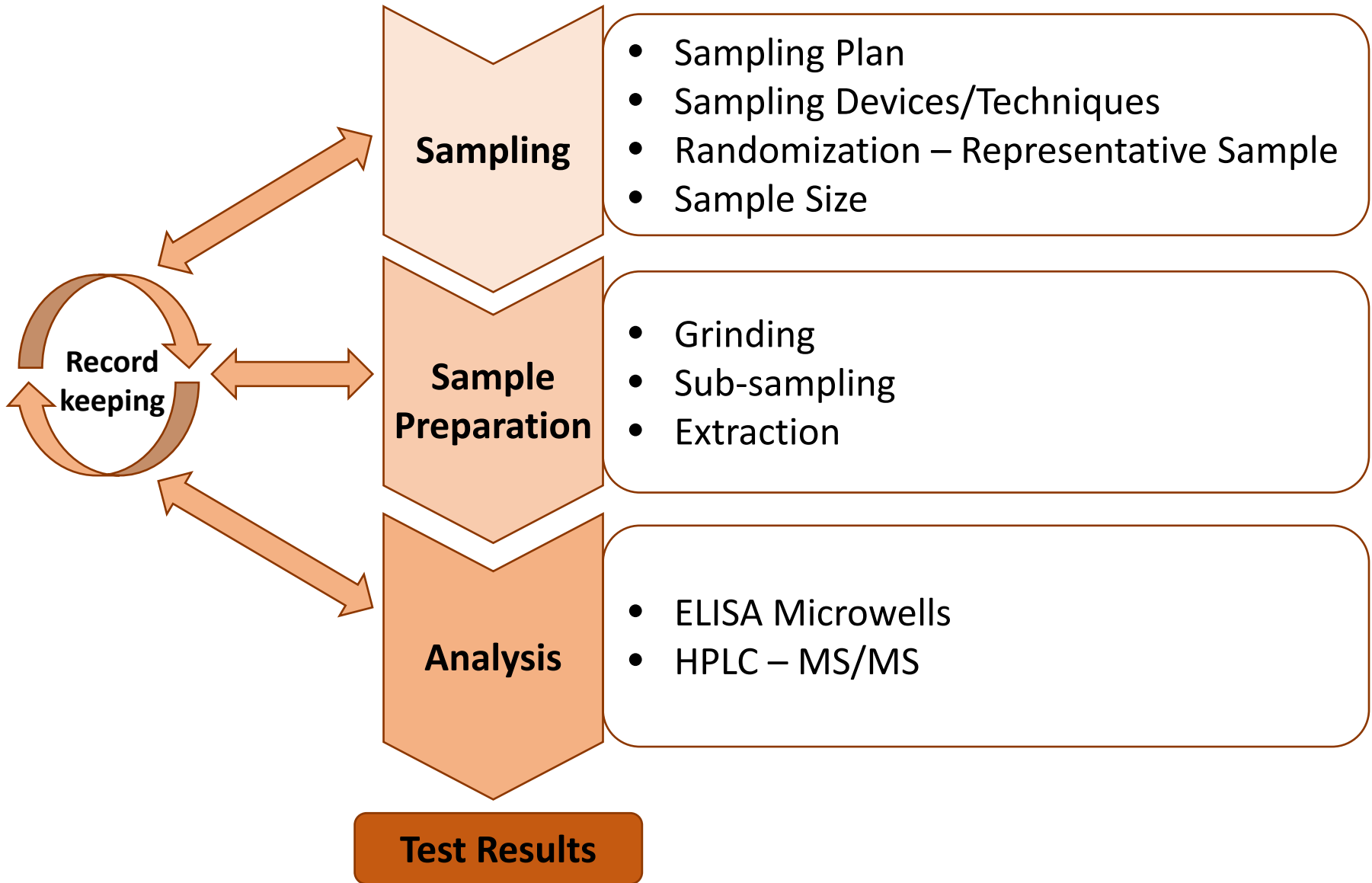
Lot ppb = Sample ppb?
ppb \leq Limit?

Sampling

Table 1. Effect of “cherry-picking” on mycotoxin test results

Mycotoxin	Commodity	Laboratory		
		Afghanistan	Austria	USA (UNL)
Aflatoxin ($\mu\text{g}/\text{kg}$)	Walnut-551	25	< LOD	< LOD
	Walnut-554	22	< LOD	< LOD
	Pistachio-612	< LOD	142	46
	Pistachio-624	< LOD	14	100
	Almond-504	14	< LOD	< LOD
	Raisin-269	18	< LOD	

Mycotoxin Analysis – Main Steps



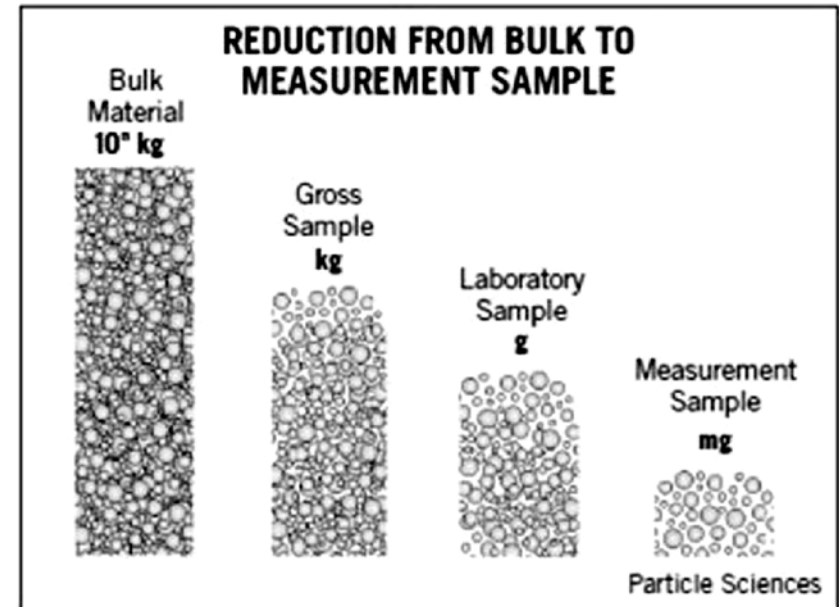
Sample Preparation

It consists of three steps:

- ✓ Grinding the lot sample
- ✓ Taking an analytical sample
- ✓ Toxin extraction

For example:

- A 10 pound sample is ground
- A 500 g subsamples is obtained
- This subsample is then mixed
 - ✓ A 50 g analytical sample is obtained





Sample Preparation

Grinding

The purpose of grinding is:

- ✓ To open up contaminated kernels and distribute the particles throughout the sample
- ✓ To increase the uniformity of the commodity
- ✓ To accelerate the process of chemical reaction/extraction

Mixing

It is performed in an attempt to “homogenize” the sample

Subsampling

Once the complete lot sample has been ground/homogenized, a smaller sample needs to be taken for the actual chemical analysis

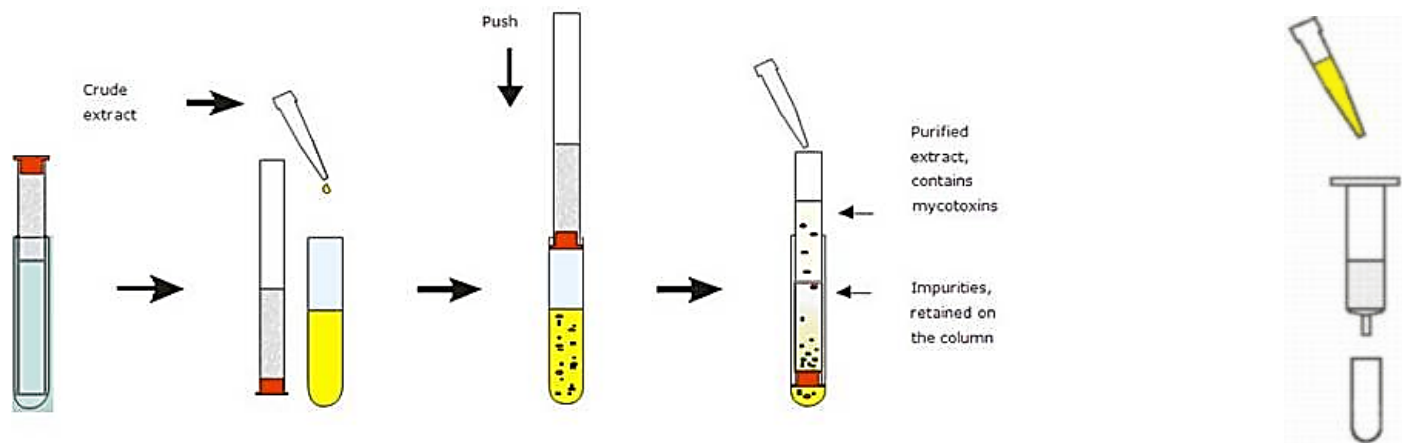
Sample Preparation

Toxin extraction

The mycotoxin is extracted by blending a solvent with the comminuted subsample

It may consist of several steps related to:

- ✓ Removing interfering compounds
- ✓ Concentrating the mycotoxins for quantification



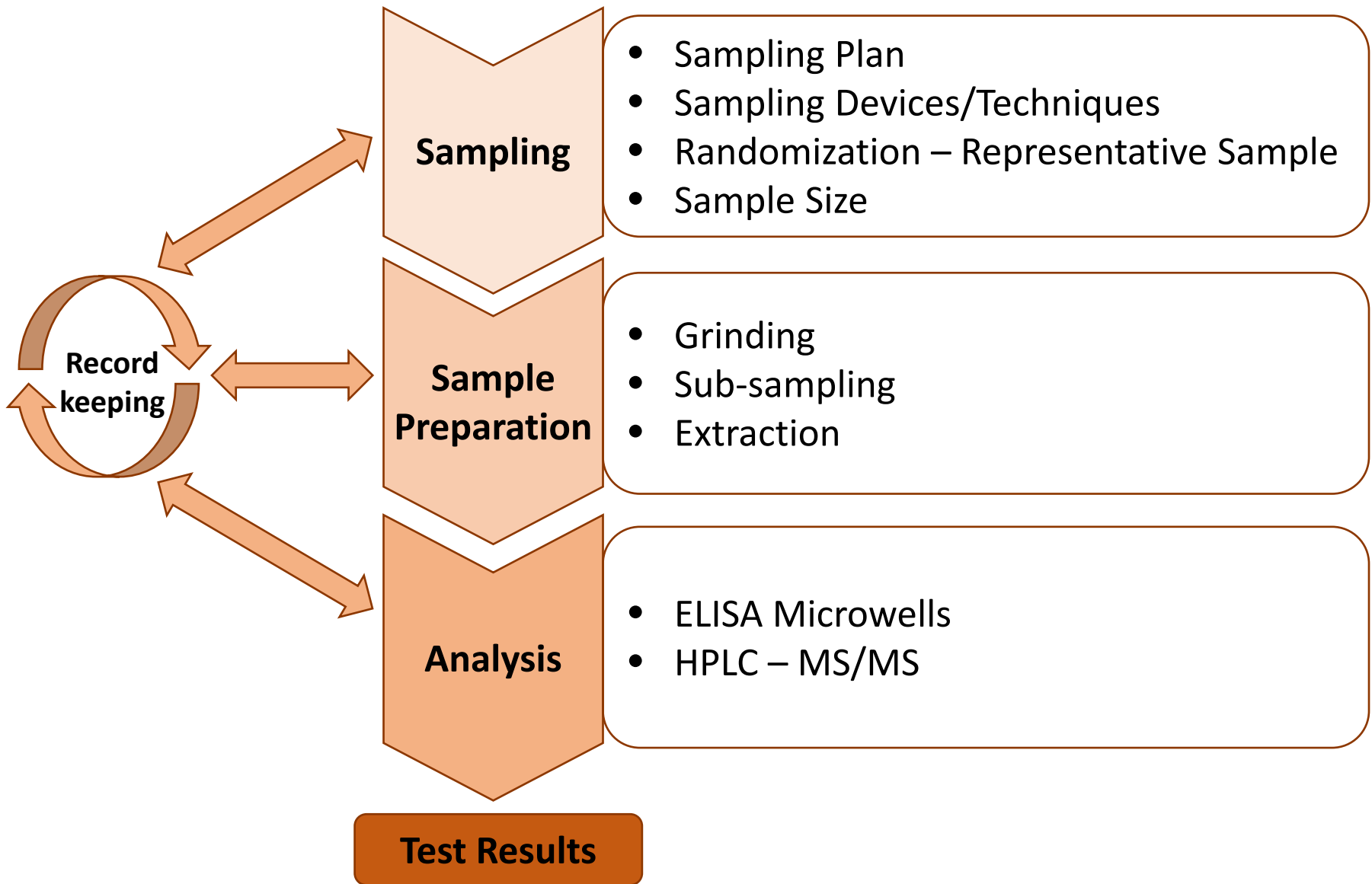
MycoSep® Working Principle – Aflatoxin Analysis by Romer Labs

Sample Preparation

Table 2. Effect of “splitting before grinding” on mycotoxin test results

Mycotoxin ($\mu\text{g}/\text{kg}$)	Commodity	Batch 1	Batch 2		
		Afghanistan	Austria	USA (UNL)	USA (KSU)
Aflatoxin	Wheat-68	5	< LOD	< LOD	< LOD
	Wheat-110	10	< LOD	< LOD	< LOD
	Walnut-551	25	< LOD	< LOD	
	Pistachio-624	< LOD	15	95	
Deoxynivalenol	Wheat-3	3500	< LOD	< LOD	< LOD
	Wheat-14	1290	< LOD	< LOD	< LOD
Ochratoxin	Raisin-296	13	< LOD		
	Raisin-302	< LOD	10		

Mycotoxin Analysis – Main Steps





Analysis

Analytical methods used in this project:

- ELISA methods – Antibody Technology
 - ✓ Romer Labs
 - ✓ Neogen Corporation
 - ✓ VICAM
- HPLC – MS/MS

Sources of bias:

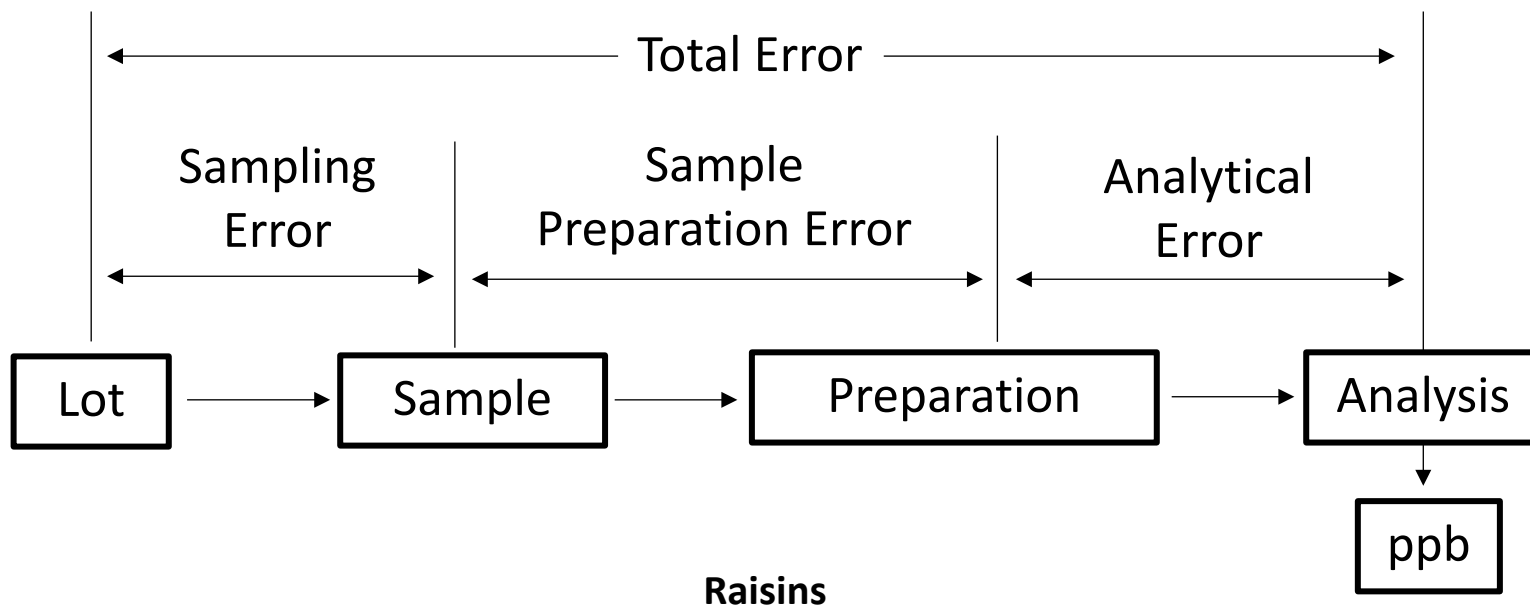
- ✓ Less than 100% of the mycotoxin may be extracted
- ✓ Other compounds may be extracted
- ✓ Mycotoxin standards may not be exact
- ✓ Instruments may not be correctly calibrated
- ✓ Antibodies among microwells may not be homogeneously distributed

Analysis

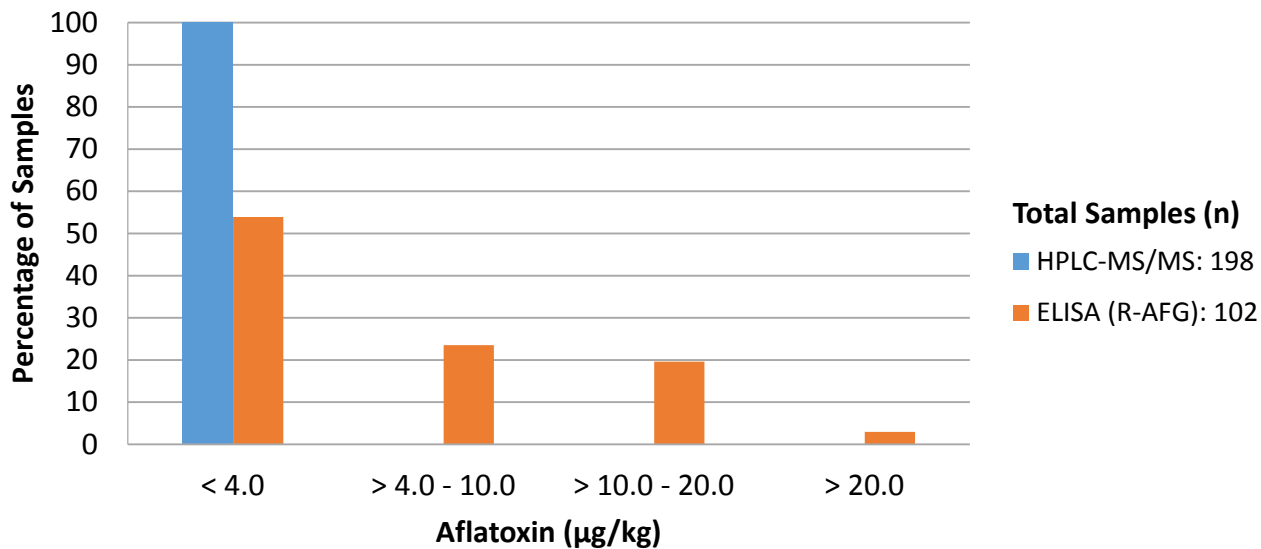
Table 3. Differences in results among analytical methods

Mycotoxin (µg/kg)	Commodity	Analytical Method		
		ELISA-Romer	ELISA-Neogen	HPLC-MS/MS
Aflatoxin	Pistachio-600	< LOD	< LOD	< LOD
	Pistachio-606	9	6	5
	Pistachio-607	< LOD	< LOD	1
	Pistachio-610	< LOD	20	< LOD
	Pistachio-611	< LOD	< LOD	< LOD
	Pistachio-612	46	29	142
	Pistachio-614	< LOD	< LOD	< LOD
	Pistachio-618	< LOD	6	0.4
	Pistachio-622	< LOD	< LOD	1
	Pistachio-624	100	95	14
	Pistachio-628	77	82	82
	Pistachio-629	< LOD	< LOD	< LOD
	Pistachio-632	27	24	14

Total Error



Raisins



Record Keeping

It is the process of recording and maintaining the history of a sample throughout the mycotoxin test procedure

Information that must be collected includes, among others:

- Sampling location, date of collection
- Sample description, type of storage
- Sample size (lot and analytical samples)
- pH of extract
- Dilutions, OD
- Final test results

It is essential to keep records to be able to link the test results with the sample and interpret its significance/importance



Concluding Remarks

Reduce the variability associated with each step of test procedure by:

- Designing an appropriate sampling plan
- Increasing sample size
- Increasing the degree of sample comminution
- Increasing subsample size
- Increasing the number of aliquots quantified
- Following the manufacturer's guidelines during test procedure

Good record keeping is essential for traceability

Ongoing staff training and development

- Reinforce proper laboratory skills/techniques



Thank you

Questions or Comments?





FEED^{THE}**FUTURE**

The U.S. Government's Global Hunger and Food Security Initiative

Mycotoxins Impacts on child growth & Development

Ahmed Kablan, PharmD, PhD.

International Nutrition and Public Health Adviser

Office of Agricultural Research and Policy

Bureau for Food Security (BFS/ARP);

USDA/ARS/Office of International Research Program

New Delhi, India March 14th, 2016

akablan@usaid.gov



Presentation overview

- 1) Overview of the current state of the scientific evidence linking Mycotoxins to child growth
- 2) What are the criteria needed to decide that Mycotoxins cause stunting
- 3) What are the possible solutions?
- 4) Conclusion



Lancet 2013

- 10 targeted interventions implemented at 90% coverage cuts stunting by 20%, mortality by 15%.
- But...“coverage rates for [many] interventions are either poor or non-existent.”
- Cost: US\$9.6 billion per annum.
- Even at 90% coverage, 80% of stunting remains!!!



Working together (i.e. Not one approach can do it all) ?

Could we achieve the 100%??



The problem-----Chronic Malnutrition

- 165 million <5 are stunted
- **Around 55%** is the percentage of child stunting in Afghanistan (range from 24.3% to 70.8%)

Several Research efforts are focusing on identifying presently unknown causes of growth retardation!!!!

Mycotoxins (e.g. Aflatoxin is one of those UNKs)

- ❑ First 1,000 days (i.e. from conception to 24 months of age), “window of opportunity to prevent stunting”
- ❑ Scaling up of 10 proved nutrition-specific interventions to cover 90% of stunted will reduce stunting by **20% ONLY?? (Lancet 2013)**



What are Mycotoxins

- A mycotoxin (from Greek (mykes, mukos) "fungus" and (toxikon) "poison") is a toxic secondary metabolite produced by organisms of the fungi kingdom, commonly known as molds. The term 'mycotoxin' is usually reserved for the toxic chemical products produced by fungi that readily colonize crops (e.g. maize, groundnuts, wheat, and many other staple foods)
- Drought stresses crops, Pest infestation also increase infection rates
- Control of toxin happen pre-harvest (e.g. Aflasafe in the case of aflatoxin) or post-harvest good agronomy practices (e.g. good drying practices and proper storage minimizing moisture).





Mycotoxin are generally of 3 origins:

- **Aspergillus**
- **Fusarium**
- **Penicillium**



Climatic conditions affect the production of mycotoxins:

- ***Aspergillus* toxins** ----Hot humid weather encourages toxin.
- ***Fusarium*** diseases are more commonly associated with cold conditions and with insect damage and wet conditions late in the growing season.
- ***Penicillium*** molds grow in wet and cool conditions and some require little oxygen



Mycotoxins linked to child growth impairment:

① Aflatoxin

- Based on Most Recent Evidence suggested from Scientific literatures

② Fumonisin

- May share a downstream pathway for impaired growth by targeting the intestinal tract and inducing environmental enteropathy (EE)?!?!?

③ Deoxynivalenol



1) Aflatoxins :

What do we know about Aflatoxins toxicity?

➤ Acute Exposure

If large doses are eaten, it will cause rapid death (e.g. Aflatoxicosis; Kenya 2004, 317 cases of reported death)

➤ Chronic exposure (Most serious thing to worry about)

➤ Chronic exposure to low doses Cause of liver cancer

➤ May Cause child stunting and low birth weights in animals and humans!!!!



Growth impacts Suggested by:

- Evidence from human and animal studies -2 key studies in human
- Current knowledge of the biological mechanisms of action of aflatoxin

How do we get exposed to Aflatoxins:

- Aflatoxin present in dried foods; human breast milk; cow milk, poultry, eggs, and meat if animals given feed with aflatoxins.
- Child exposure risk increases after weaning
- Dependence on single commodity with little diet diversity increase the risk of exposure significantly



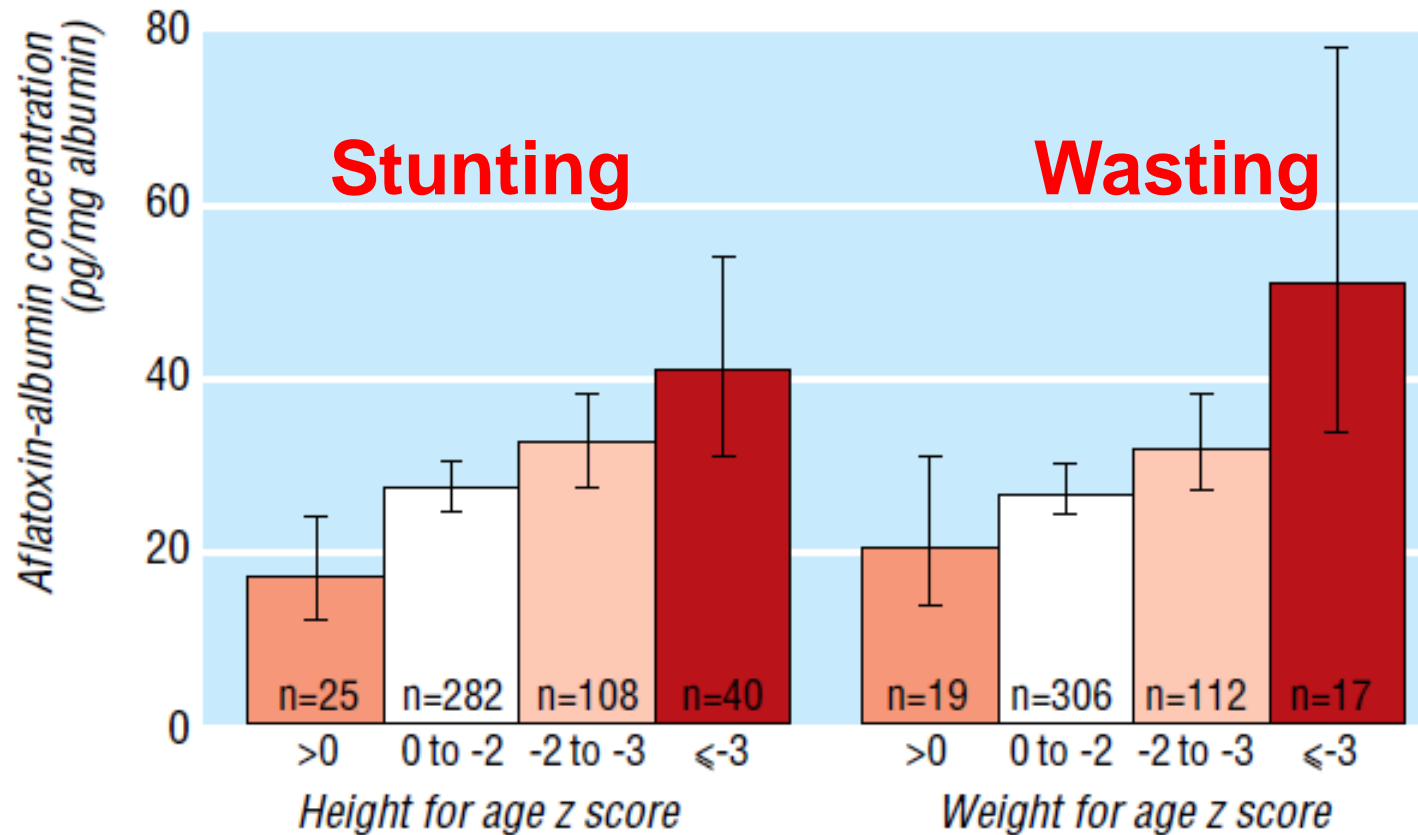
E.g. Studies linking aflatoxin to growth impairment in children-Just an example of the evidence!!

Type of study	Results	Nation & study
Aflatoxin measurements in stored flour, rural homes	Stunting, underweight, & wasting associated with higher AF levels in flour	<i>Kenya</i> (Okoth & Ohingo 2004)
Cross-sectional: AF-alb levels in maternal, cord, child blood	Stunting & underweight associated with higher AF-alb levels in these fluids	<i>Togo, Benin, United Arab Emirates, The Gambia</i> (Gong et al. 2002*, Abdulrazzaq et al. 2004, Turner et al. 2007)
Longitudinal: AF-alb levels in children's blood	Reduced height gain in 8 mos associated with AF-alb levels	<i>Benin</i> (Gong et al. 2004)
AFM1 in mothers' breastmilk	Lower length at birth & in infancy associated with AFM1	<i>Iran</i> (Sadeghi et al. 2009, Mahdavi & Nikhniaz 2010)

*Dose-response relationship between AF-alb & HAZ, WAZ



- Gong et al (BMJ, 2002) showed that **stunting** and **weight for age** was inversely related to aflatoxin levels in Gambia. Jolly and colleagues (Peanut Innovation Lab) have shown the same in Ghana.





FUM and stunting

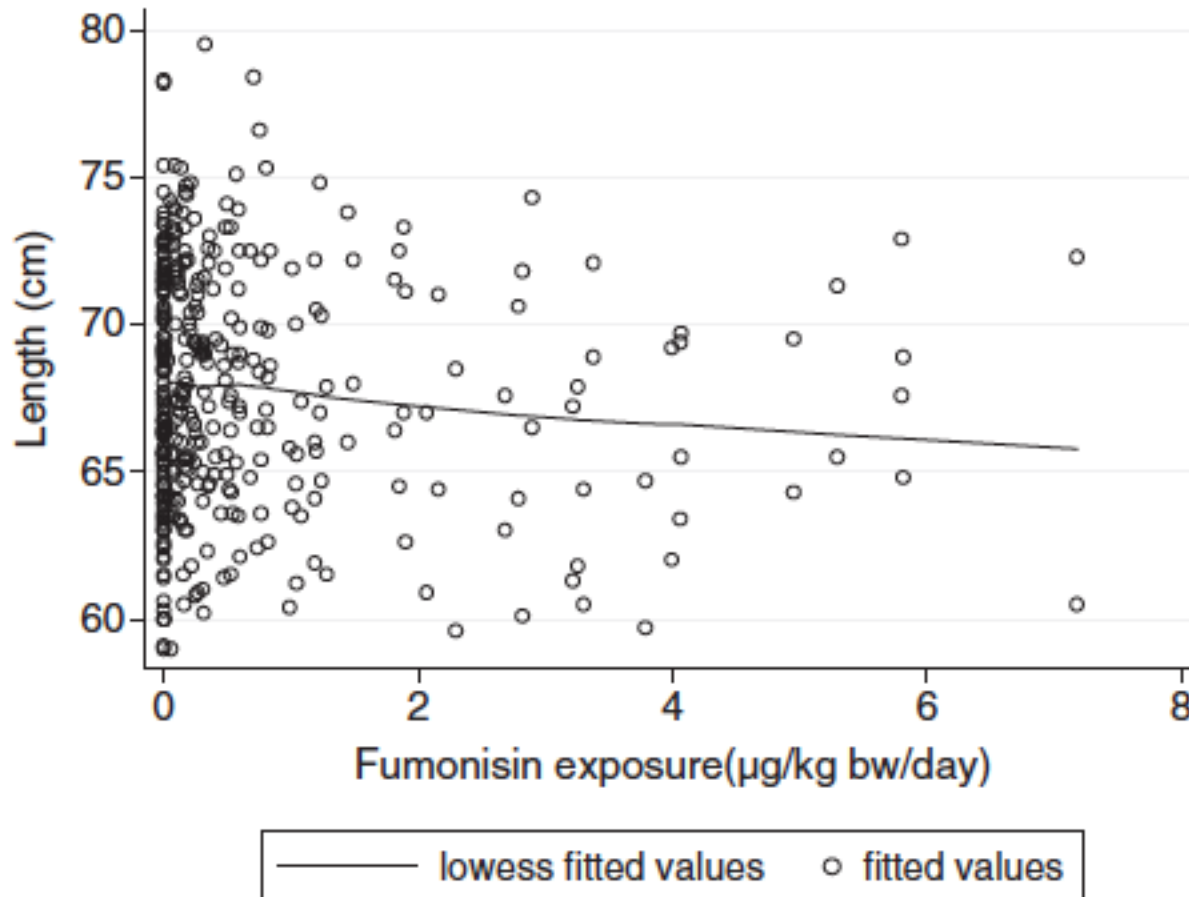
- FUM B is the most potent of the FUM toxins and may cause decreased expression of local pro-inflammatory cytokines and disruption of sphingolipid metabolism
- Biomarker to for FUM relatively recently identified and been validated (Gong Y et al, 2008)



- FUM B has been associated with renal tumors, self-reported abdominal pain and diarrhea , esophageal cancer, increased risk for neural tube defects , and retarded growth
- Positive correlation had been found between high maize consumption and **HIV transmission** ----- FUM exposure?? (FUM effect on cell membrane integrity??)



- Dilkin et al (2003) found that pigs **fed FUM alone or FUM and AF** combined had a decrease in food consumption and body weight-----Synergistic effect????
- Kimanya et al (2010) found that children with **FUM intakes (CF/Weaning food) >** than the provisional maximum tolerable daily intake (PMTDI) were significantly shorter and lighter than children with FUM intakes less than the PMTDI



Relationship between FUM exposure from complementary food & Length in infants @ 12 months



DON and Stunting

- Deoxynivalenol (DON or vomitoxin).
 - DON is a trichothecene mycotoxin produced by some *Fusarium* species.
 - Can cause nausea, diarrhea, and vomiting.
 - DON is able to cross placenta and reduce growth of unborn children.



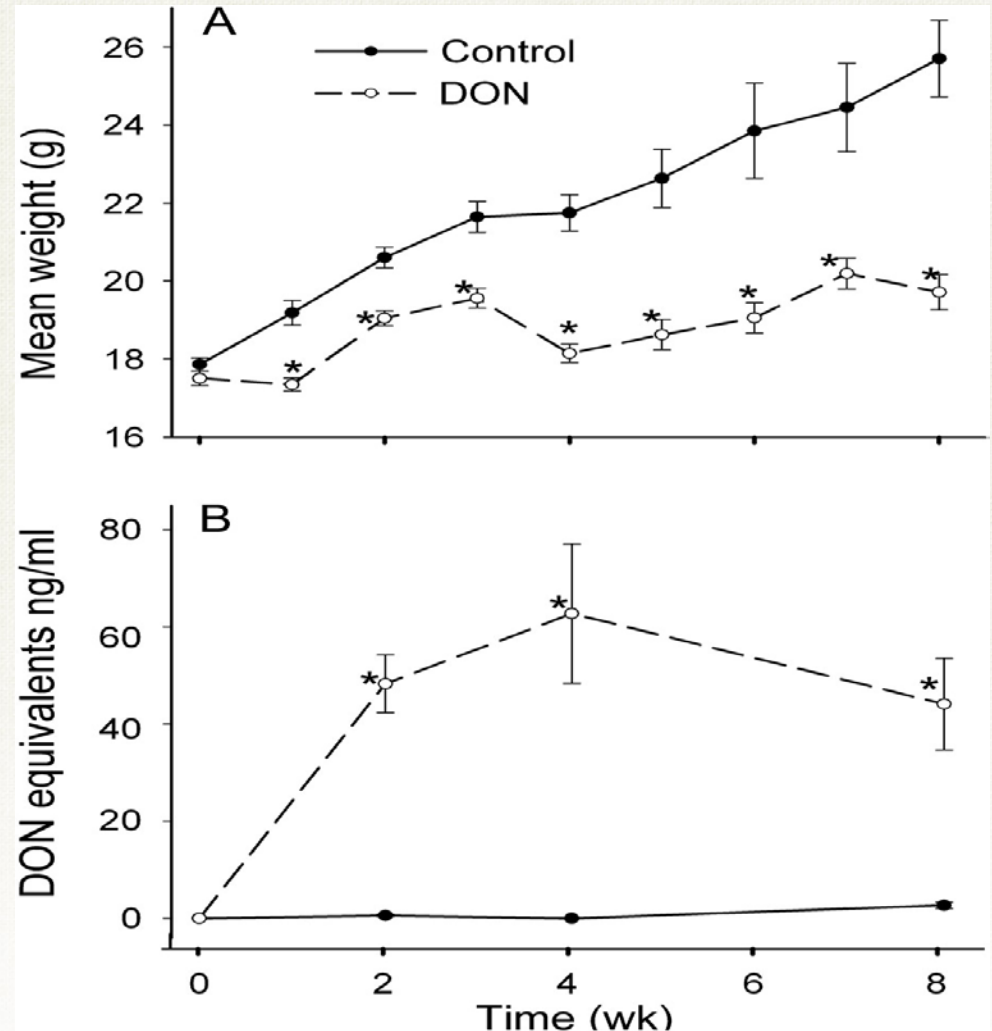
- DON effect on Child stunting is still under investigation
- It is likely that DON has a negative effect on growth because of decreased food intake and reduced weight gain that has been observed in animal studies



- Knowledge we have from animal studies---In 1995 Rotter et al found that pigs fed grain contaminated with DON had a 20% lower feed intake and a 13% lower weight gain than the control group
- Amuzie and Pestka (2010) found that DON intake in mice induced a decrease in circulating levels of IGF-1 an important mediator of the growth hormone axis



DON consumption reduces weight gain and increases plasma DON in mice.



Chidozie J. Amuzie, and James J. Pestka *Toxicol. Sci.* 2009;113:412-421

© The Author 2009. Published by Oxford University Press on behalf of the Society of Toxicology. All rights reserved. For permissions, please email: journals.permissions@oxfordjournals.org



How does Mycotoxins cause stunting? **Exact Mechanism is still missing**; however several has been proposed:

- 1) **Immunomodulation associated with aflatoxin exposure** (Bondy and Pestka, 2000; Turner et al., 2003) ---cause recurrent infections in children, which can lead to growth impairment (Gong et al., 2008)
- 2) **Changes in intestinal integrity** (possibly in part resulting from immunomodulation) could make hosts more vulnerable to intestinal foreign microbes (Gong et al., 2008)
- 3) **Downregulation of genes associated with energy production and fatty acid metabolism** (Yarru et al., 2009)
- 4) **Impairment of protein synthesis and the inability to mobilize fat** (Kocabas et al., 2003)
- 5) **Changes in hepatic metabolism of vitamins and micronutrients** (Schaeffer and Hamilton, 1991).

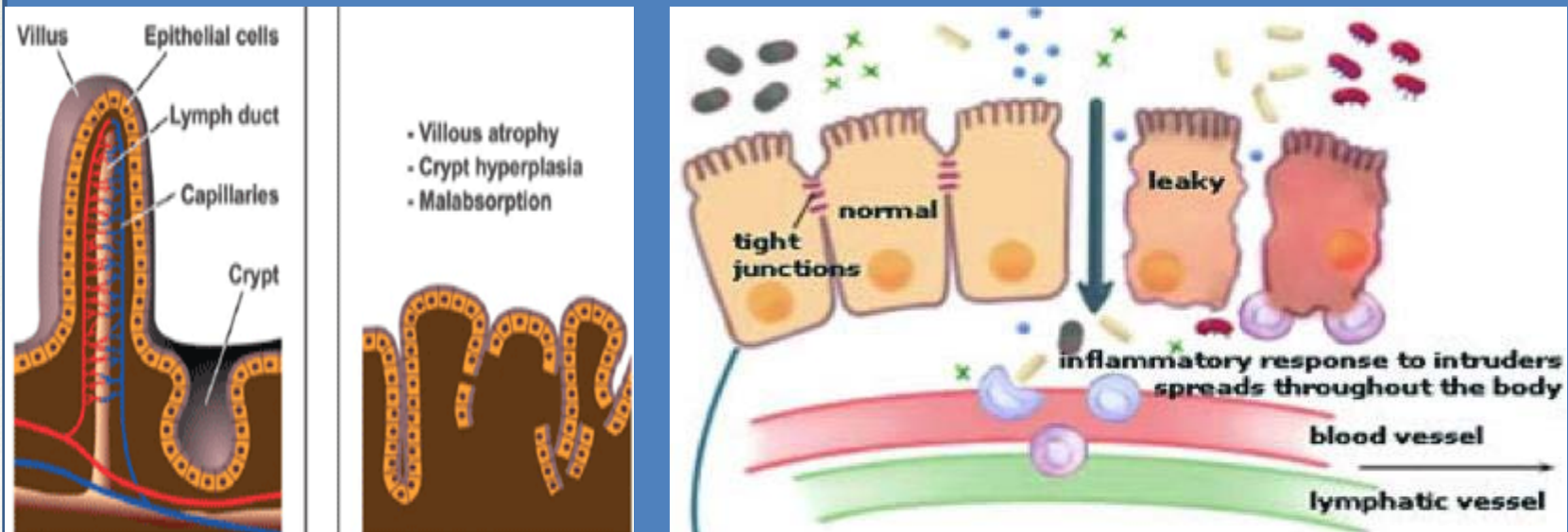


AF

DON

FUM

Aflatoxin together with DON and fumonisin, might lead to environmental enteropathy



Impaired Growth



Interventions to reduce aflatoxin risk

R
e
d
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c
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E
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➤ Preharvest

- Good agricultural practices
- Genetically enhancing plants' resistance
- Biocontrol
- Biotechnology/breeding



➤ Postharvest

- Improved sorting, drying, food storage
- Crops not prone to aflatoxin (e.g. Soybean)

M
i
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➤ Dietary

- Improved dietary diversity
- **Dietary enterosorbents**
- **Dietary chemoprevention**
- Curcumin
- Compounds in cruciferous & Allium vegetables
- Green tea polyphenols

➤ Hepatitis B vaccine:

- Aflatoxin consumption in HBV+ patients increase risk of Liver cancer

Wu F, Khlangwiset P (2010). "Health economic impacts and cost-effectiveness of aflatoxin reduction strategies in Africa: Case studies in biocontrol and postharvest interventions." *Food Addit. Contam* 27:496-507.





What does this mean?

- **Eliminating stunting & malnutrition** will require provision of adequate and diverse diets; removing environmental contamination (e.g. **Aflatoxin**) ; preventing infectious diseases.
Why these?
- A systematic review of nutrition programs: very best programs only deal with ~ **1/3rd of stunting** at best
- Stunting is strongly related to foodborne **toxins (such as **Aflatoxin**)**, etc.



Conclusions

- Aflatoxin relation with Stunting?  It is strongly associated with it and likely a cause; other Mycotoxins still under investigation

- What is needed next?  Controlled experimental studies urgently needed.





➤ Will Aflatoxin reduction improve the health problems associated with stunting e.g. cognition problems?



We don't know; we need to test this

➤ Should we wait to take an action for more evidence ?

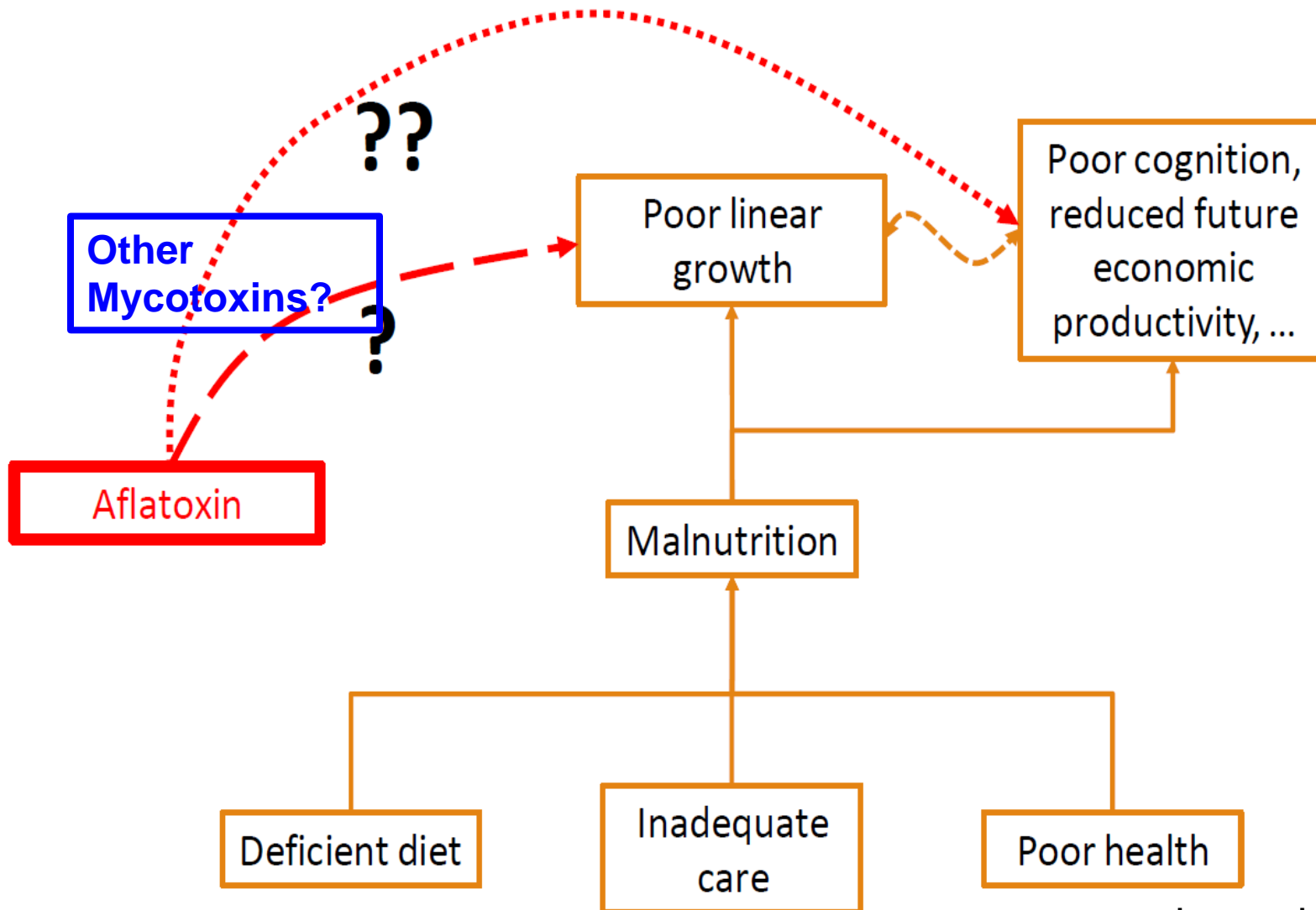


Absolutely not; we have enough evidence from animal and human studies and we need to take actions urgently.





Question??





FEED THE FUTURE

The U.S. Government's Global Hunger and Food Security Initiative



www.feedthefuture.gov



@Feedthefuture

Thank you



Food additives:

- “Enterosorbents” trap aflatoxins in the gut
- E.g. Calcium montmorillonite clay (marketed as NovaSil)
- Evidence on efficacy:
 - Ghanaian adults given a placebo, either a 1.5- or 3-gram clay capsule; Daily for three months;
 - Net reduction in serum aflatoxin levels of 21% and 24%.

Remaining questions and concerns:

- Effect large enough to reduce negative effects on linear growth?
- To what extent does clay also bind micronutrients and lead to micronutrient deficiencies?



Chemopreventive agents:

- Chlorophyllin (a derivate of chlorophyl) and oltipraz (an antischistosomal drug);
- Intervene in the biochemical pathway linking liver cancer to aflatoxin exposure;
- Whether effective in stunting pathway is unknown.

Important concern for use of both enterosorbents and chemopreventive agents:

- Should not be interpreted as a substitute for good crop agronomy
- Should not unintentionally encourage the use of foods not fit for human consumption.

“International Conference on Food Quality
and Safety”



March 14-16, 2016 – New Delhi (India)

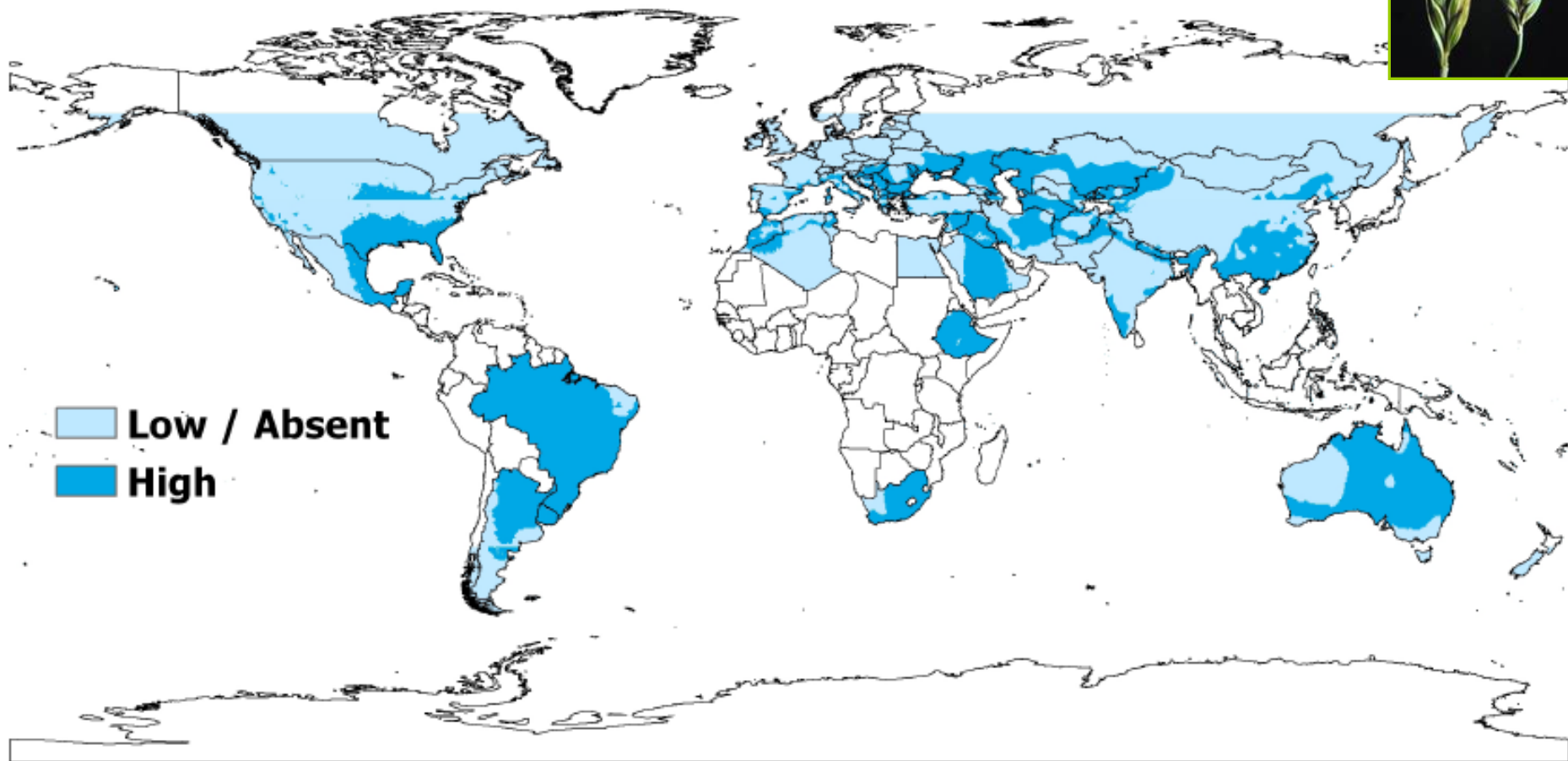
MYCOTOXIN REGULATIONS



Antonio F. Logrieco

*Institute of Sciences of Food Production (ISPA)
National Research Council (CNR)*

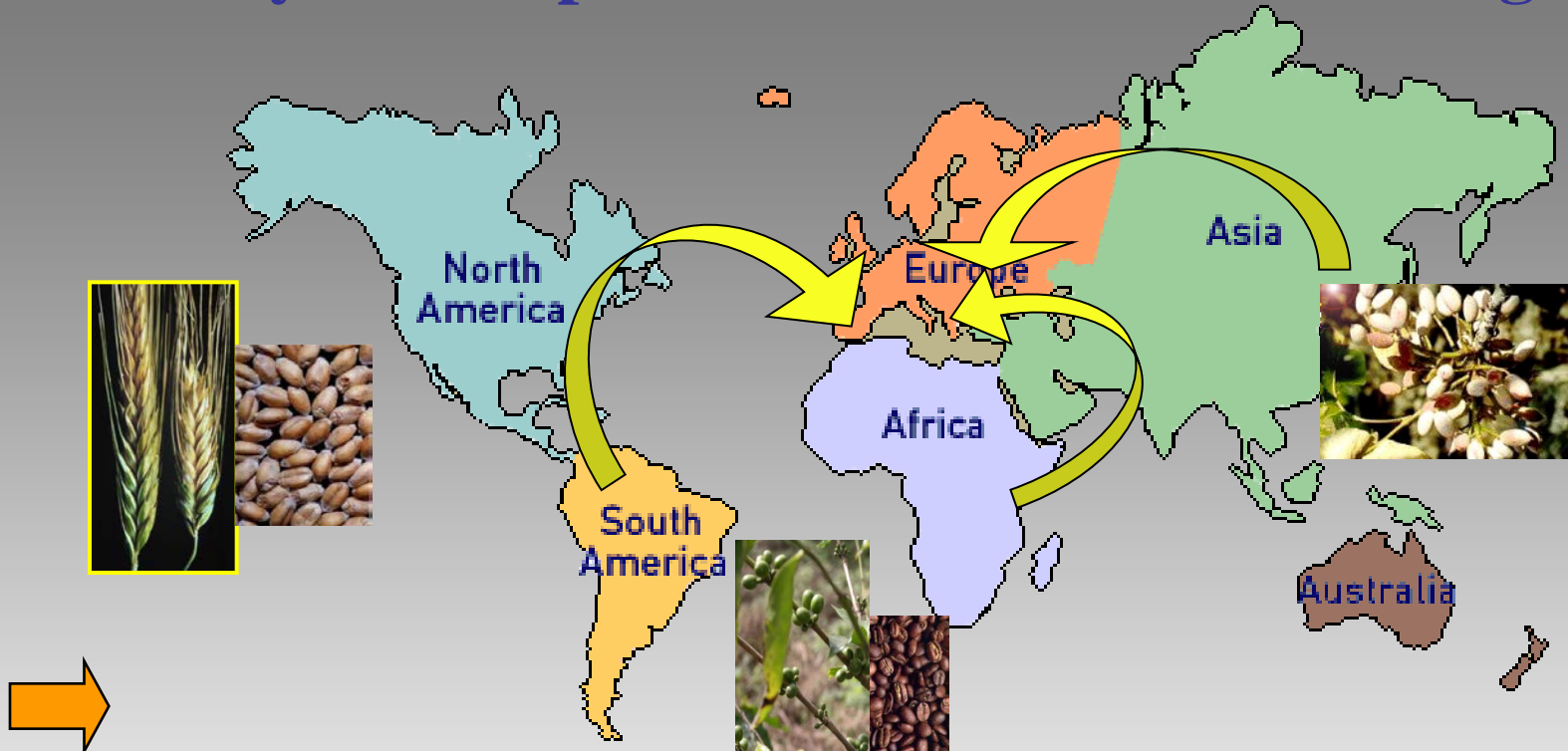
Map of DON risk for wheat



Low / Absent
High

Battilani and Logrieco, 2012

Mycotoxin problems due to trade exchanges



Imported products with high risk of mycotoxin contamination:

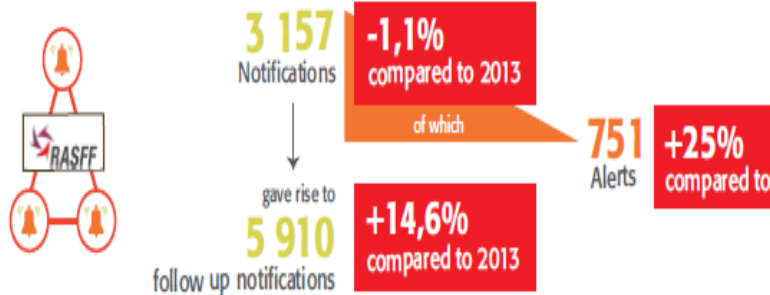
- **maize**, (fumonisins and aflatoxins) from all continents
- **cereals** (deoxynivalenol, ochratoxin A) mostly from north and south America
- **coffee**, (ochratoxin A) mostly South America & Africa
- **pistachio nuts**, (aflatoxins) mostly from North Africa & Asia
- **Peanuts & other nuts**, (aflatoxins) mostly North, South America & Africa
- **Spices** (aflatoxins) mostly from Asia & Africa



RASFF 2014 NOTIFICATIONS



RAPID ALERT SYSTEM FOR FOOD AND FEED (RASFF) Annual Report 2014



Mycotoxin notifications: 54
Border rejections: 280
Information for attention: 44
Alert: 5
Information for follow-up: 383

NOTIFICATIONS BY PRODUCT CATEGORY



NOTIFICATIONS BY HAZARD

PATHOGENIC MICRO-ORGANISMS

782



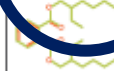
PESTICIDE RESIDUES

435



MYCOTOXINS

383



HEAVY METALS

285



COMPOSITION

216



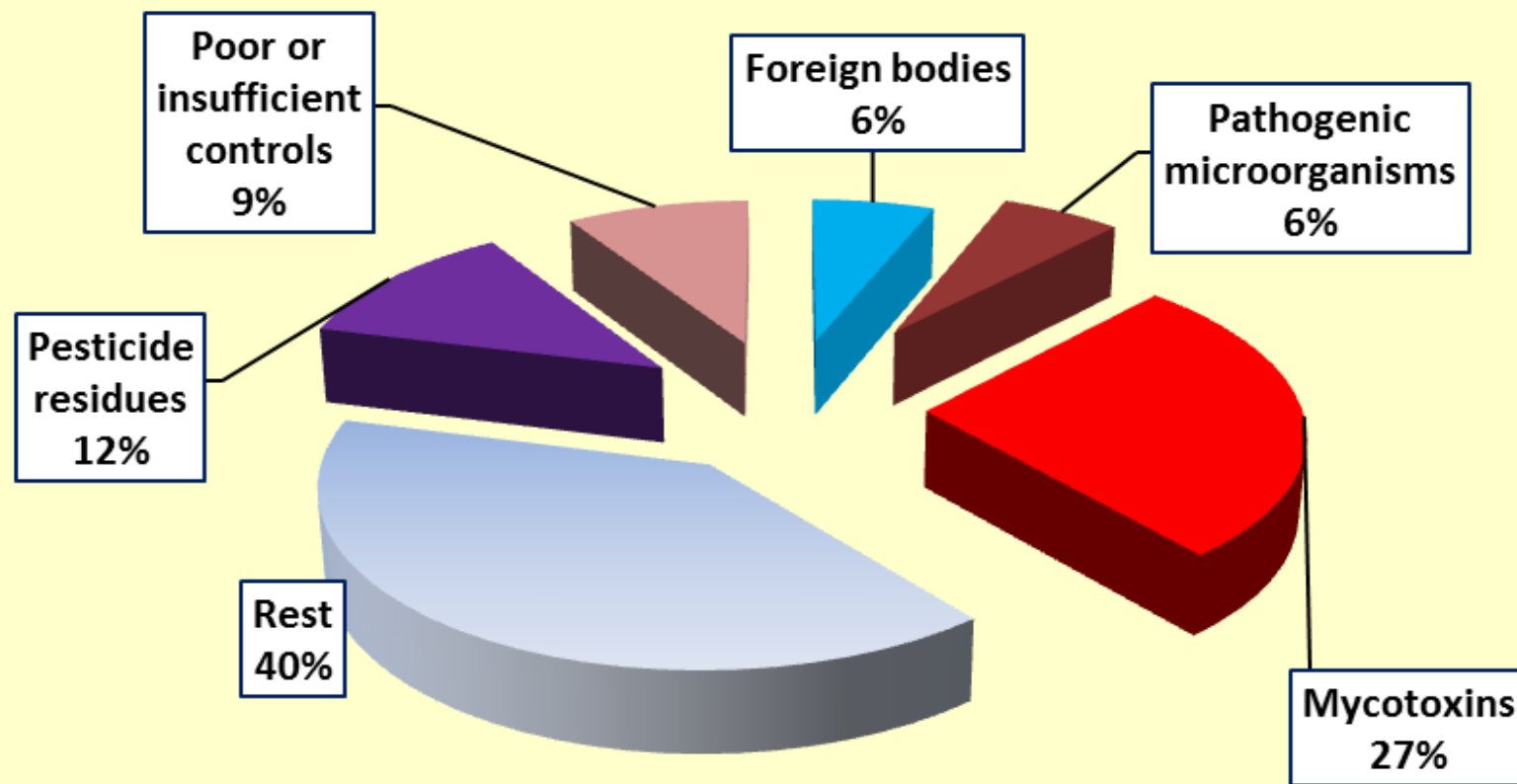
FOOD ADDITIVES AND FLAVOURINGS

132



Food and Feed

RASFF 2011: Border rejection notifications



Border rejection notifications 2011
(hazard category)

AFLATOXINS ARE THE LARGEST CHALLENGE AMONG MYCOTOXINS

Table 2 - Notifications on mycotoxins in food and feed

Hazard	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013
Aflatoxins	839	946	801	705	902	638	649	585	484	341
Deoxynivalenol (DON)				10	4	3	2	11	4	8
Fumonisin	14	2	15	9	2	1	3	4	4	7
Ochratoxin A	27	42	54	30	20	27	34	35	32	54
Patulin		6	7		3					
Zearalenone			1	6	2				4	
Total mycotoxins	880	996	878	760	933	669	688	635	528	410



Rapid Alert System for Food and Feed

Most aflatoxin-related notifications are linked to nuts and dried fruit



Table 5 – Aflatoxin notifications for certain products under reinforced checks regime

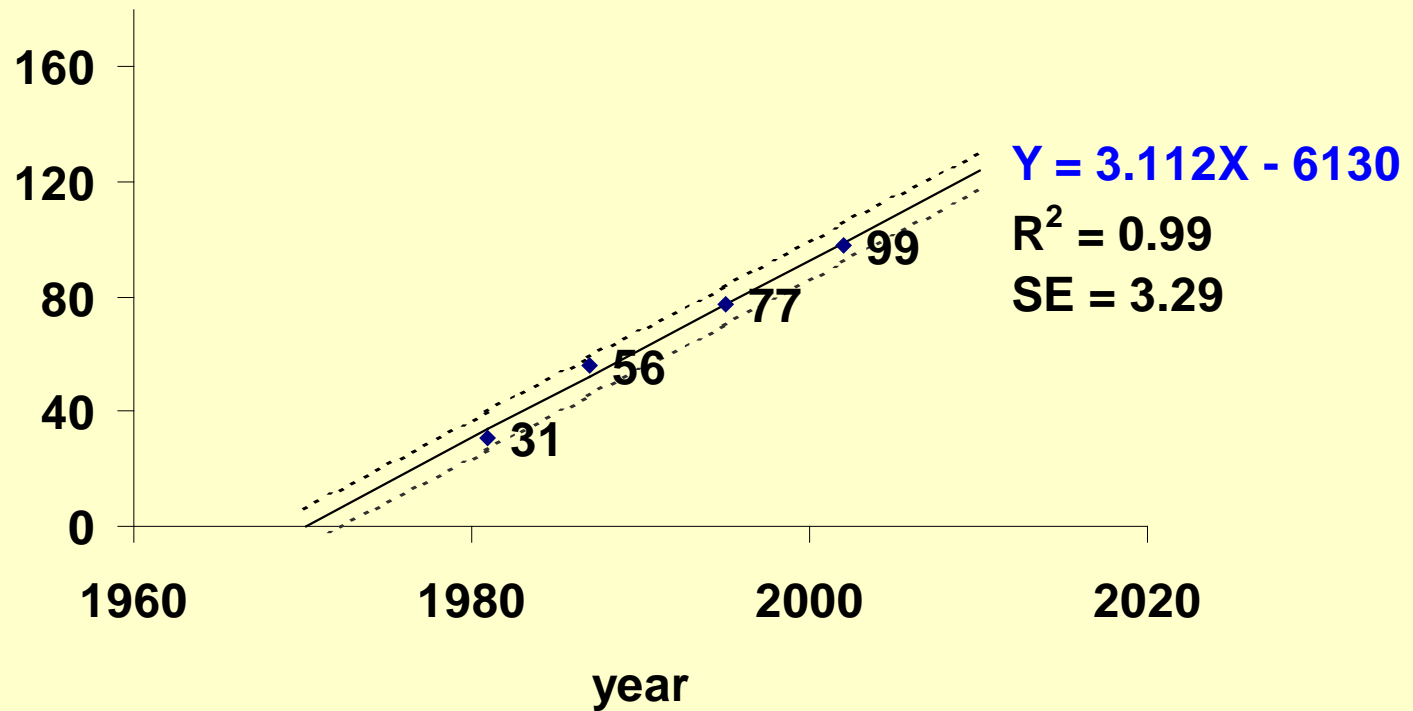
Product	Number of notifications in 2012	Number of notifications in 2011
Peanuts from India	88	133
Peanuts from Argentina	13	40
Pistachios from Iran	20	38
Pistachios from Turkey	13	41
Hazelnuts from Turkey	4	17
Spices from India	24	41
Dried frigs from Turkey	135	75

source: RASFF 2012 Annual Report

Mycotoxin - regulating countries

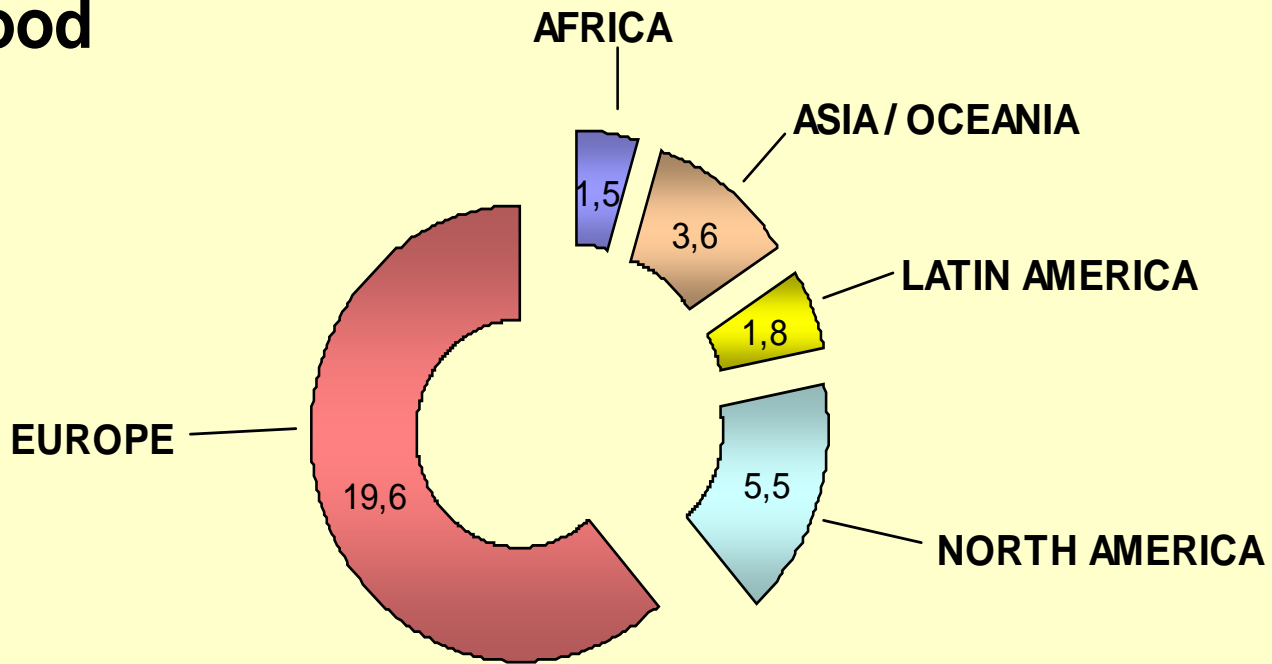
1981 - 1987 - 1995 - 2003

countries



Number of mycotoxin regulations per country

Food

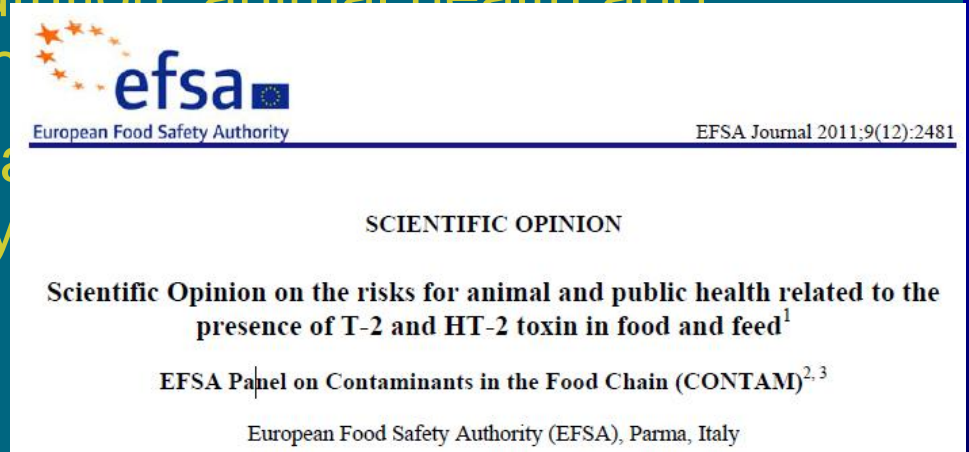


Factors influencing mycotoxin regulations

- **Availability of toxicological data**
- **Availability of survey analytical data**
- **Availability of methods of sampling and analysis**
- **Trade contacts with other countries**
- **Sufficiency of food supply**

EFSA and its mission

- EFSA: the EU's scientific risk assessment body on food and feed safety, nutrition, animal health and welfare, and plant health
- EFSA panels, in cooperation with Member States, issue scientific opinions on many topics
- Opinions published on website and in EFSA journal



Mycotoxin regulating countries in Europe

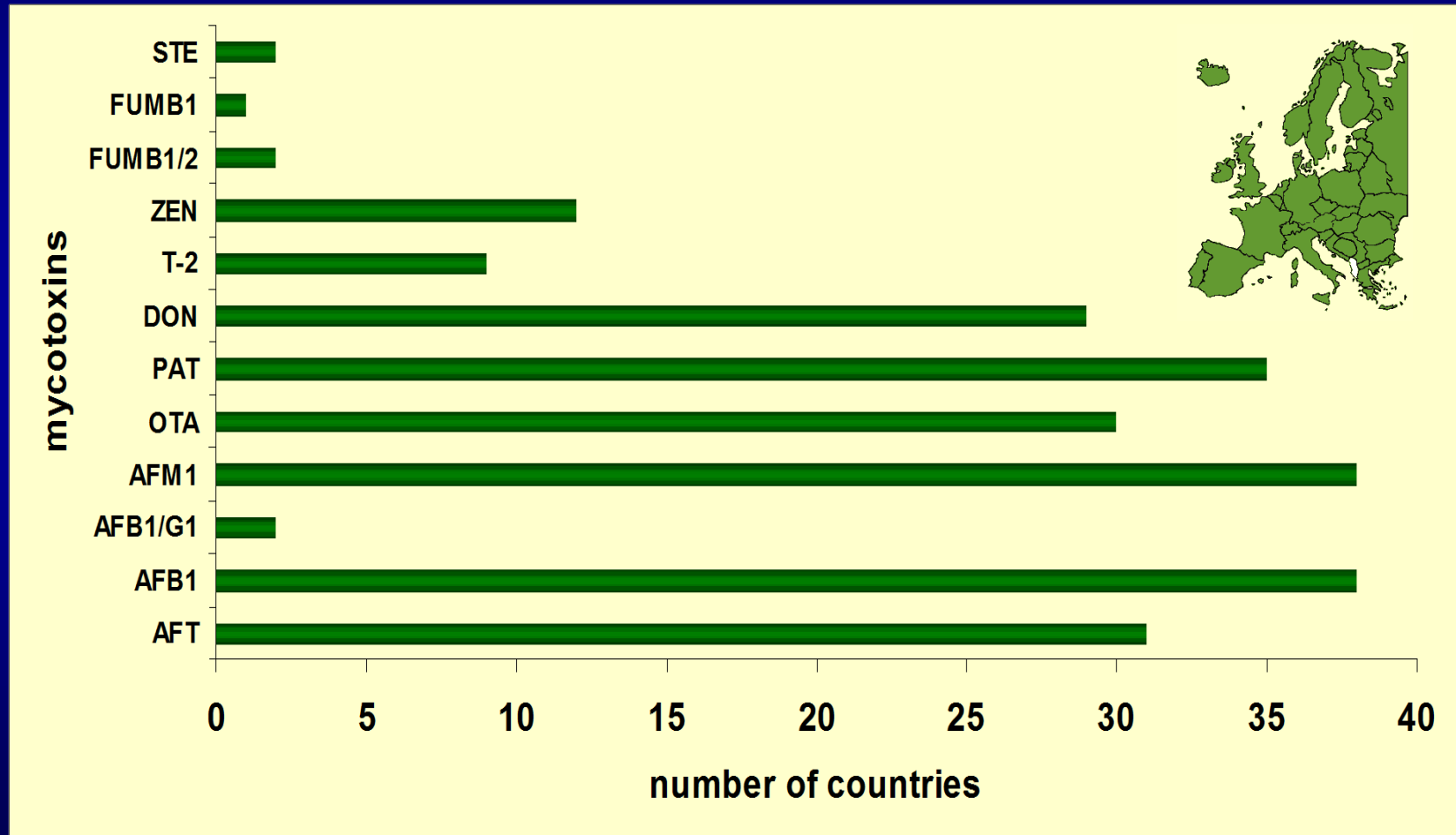
(FAO FNP 81, 2004)

- 39 nations with known regulations (99% of inhabitants of the region)
- EU harmonized limits exist for aflatoxins, ochratoxin A, patulin, zearalenone, deoxynivalenol and fumonisins
- EU regulations expected for T-2 and HT-2 toxins in foods, and for toxins in baby foods and feeds
- Most detailed: several new EU member states



Europe: mycotoxins regulated in food

(FAO FNP 81, 2004)



Mycotoxin regulating countries in Asia/Oceania

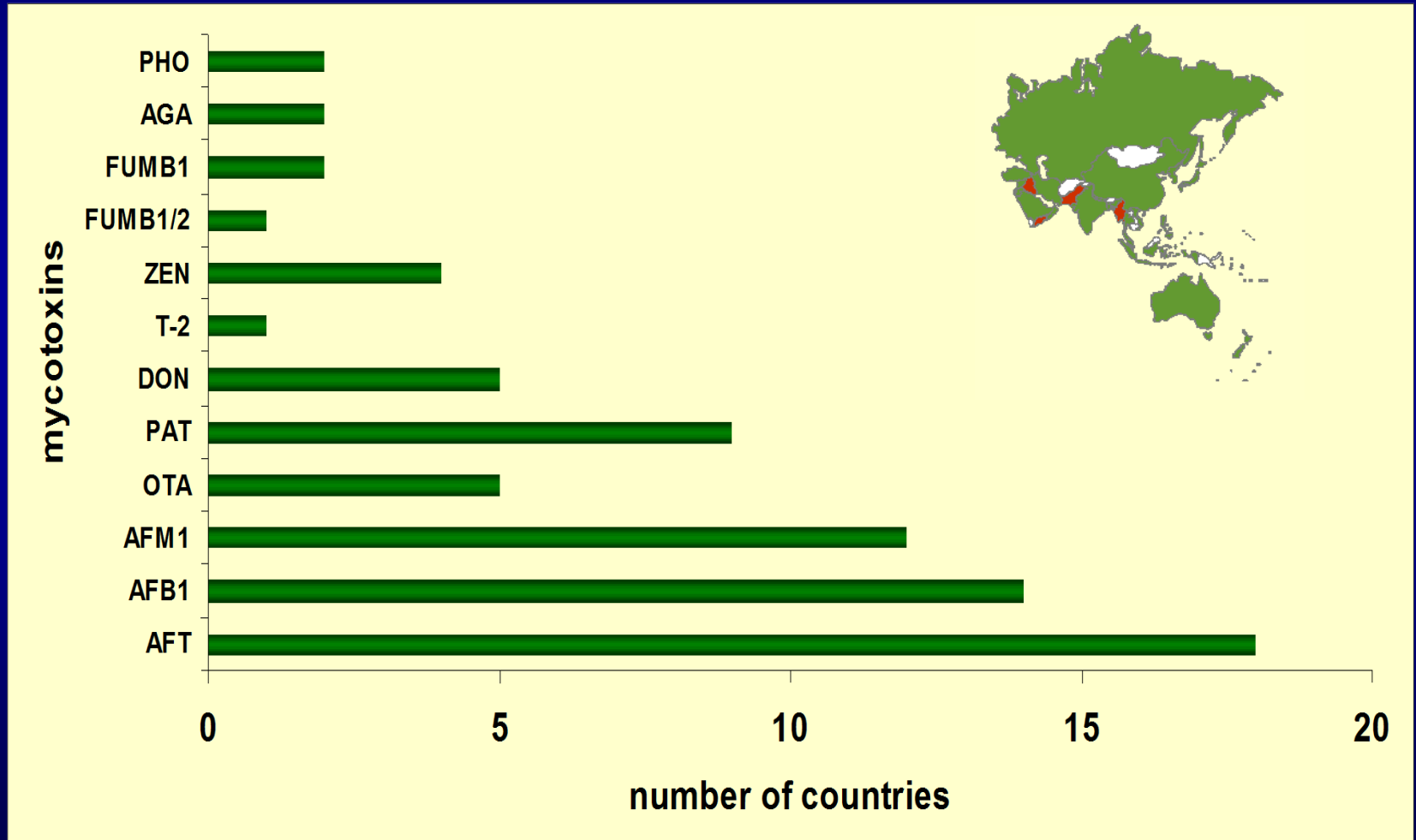
(FAO FNP 81, 2004)

- 26 nations with known regulations (89% of inhabitants of the region)
- Regulations for total aflatoxins dominate in food, regulations for AFB₁ dominate in feed
- Harmonized regulations in Australia & New Zealand
- Most detailed : China and Iran



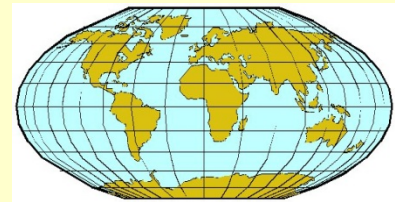
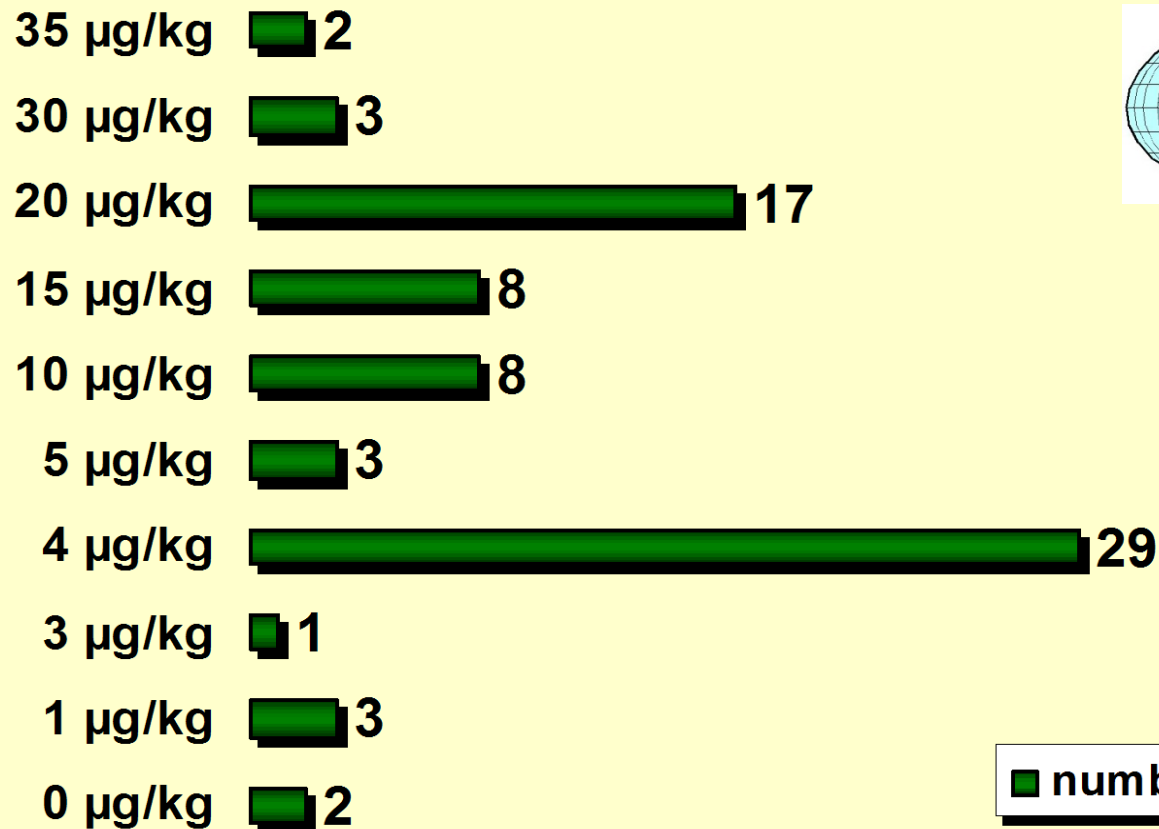
Asia/Oceania: mycotoxins regulated in food

(FAO FNP 81)



Total aflatoxins in food

(FAO FNP 81, 2004)



■ number of countries

Ochratoxin A in cereals and cereal products

(FAO FNP 81, 2004)

50 µg/kg 3

30 µg/kg 1

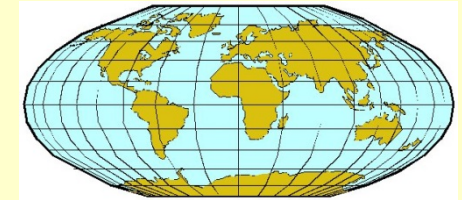
20 µg/kg 1

15 µg/kg 1

10 µg/kg 1

5 µg/kg 29

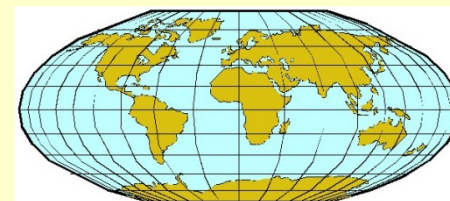
3 µg/kg 1



■ number of countries

DON in wheat (flour) and other cereals

(FAO FNP 81, 2004)



2000 $\mu\text{g}/\text{kg}$ 1

1200 $\mu\text{g}/\text{kg}$ 2

1100 $\mu\text{g}/\text{kg}$ 1

1000 $\mu\text{g}/\text{kg}$ 6

750 $\mu\text{g}/\text{kg}$ 28

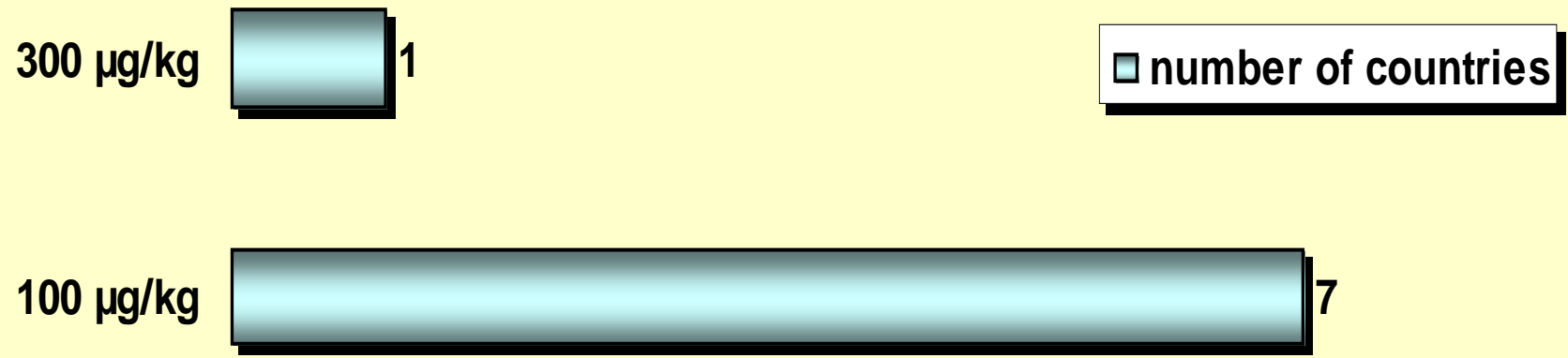
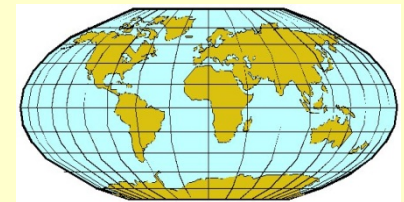
700 $\mu\text{g}/\text{kg}$ 3

300 $\mu\text{g}/\text{kg}$ 1

■ number of countries

T-2 toxin in cereals and cereal flours

(FAO FNP 81, 2004)

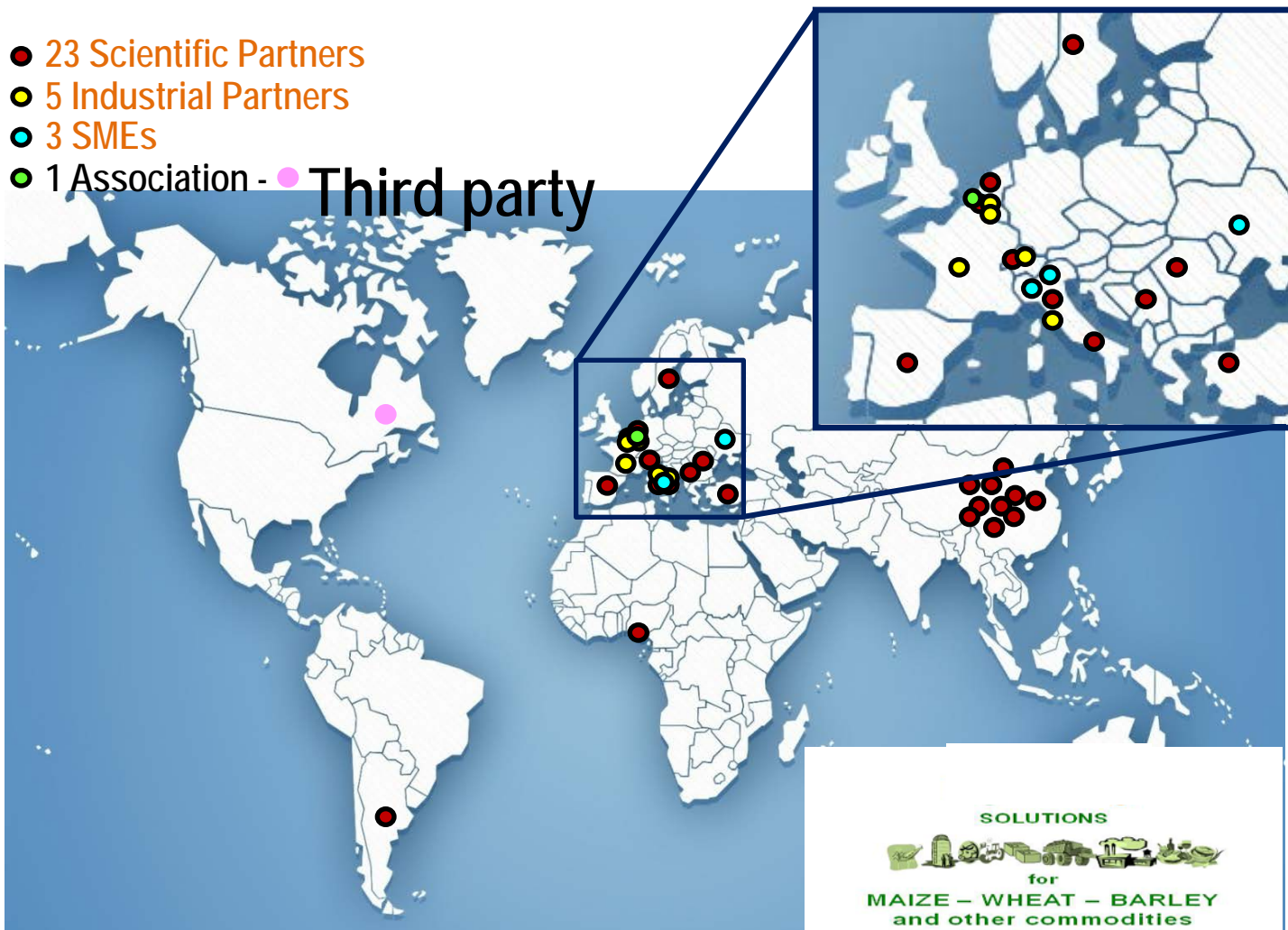




Integrated and innovative key actions for mycotoxin management in the food and feed chain

- 23 Scientific Partners
- 5 Industrial Partners
- 3 SMEs
- 1 Association - ● Third party

Third party



SOLUTIONS
for
MAIZE - WHEAT - BARLEY
and other commodities



ISTITUTO DI SCIENZE
DELLE PRODUZIONI
ALIMENTARI

**Thanks for your
attention!**

Antonio F. Logrieco

e- mail: antonio.logrieco@ispa.cnr.it



CONTROLUNION

Food Safety and International Certifications

What clients want and regulations require

Afghanistan 2016

The Agenda

- Control Union Profile
- Food Safety
- International Market Requirements
- Certifications - Options, Process and Costs
- Lab testing
- Questions and Discussion

Control Union's Global Network

- Founded in 1920 – Holland
- Globally accredited Inspections, Certifying Body (CB) & 26 Labs
- 72 countries
- 200+ certifications - 35 touching the agriculture
 - Food Safety (HACCP, ISO and Global GAP)
 - Sustainable value chains – origin and packaging (traceability)
 - Environmental
 - CoC (Chain of Custoday)
 - Social Metrics – Fair Trade, Fair Choice and GRASP
- Kabul Office Opened in 2014

What is Food
Safety and
why is it
important ?



Food Safety and Private Sector

- Food safety is a concept that food will not cause harm to the consumer when it is prepared and/or consumed – Domestic & International
- Governments establish food safety standards for domestic and imported foods.
- Food safety cannot be achieved only with government regulations and inspection – Private Sector must standardize handling of foods at all points in the food chain, from production to consumption.
- Private sector must understand their role and accept the responsibility of their actions – their responsibility to provide safe, wholesome foods to the customer – Domestic & international.
- The private sector has a responsibility to maintain the quality and reputation of the company's products and or services.



WHO is your buyer?

WHAT Does Your Buyer WANT ?

Certification

- Certifications are ALL commercial standards
- Third-party certification = VERIFICATION
- Independent assessment declaring that specified requirements pertaining to a product, person, process or management system have been met.
- 138 Global Certification Bodies – Not all CBs are accredited for every certification you need
- **What does your market legally require to import !**
- **Ask your clients questions !**
- **ASK your Certification Body questions !**



- International standards are recognized = CREDIBILITY
- Contribute to a national Afghan brand for quality food
- Improve company performance
- Build confidence
 - Employee
 - Stakeholders
- Attract Investment
- Attract buyers = New opportunity
- Increase REVENUE

PREPARE for Certification

- Be realistic about the benefits and costs of implementing quality systems. CHEAP = EXPENSIVE !
- COMMIT the time and resources to develop a framework that will support business continuity and growth.
- Certification should become ‘business as usual’



What Certification does your business need ?

1. Who is your buyer ?
2. What country is your buyer exporting to ?
3. What is your buyer requesting?
4. What is your business plan ?
5. Do you have an export strategy ?
6. Plan Ahead
 1. Year 1 = HACCP
 2. Year 2 = ISO 22000
 3. Year 3 = Social Metric Standard (Retail vs Wholesale)

The BIG Options



GLOBALG.A.P.
The Global Partnership for Good Agricultural Practice



Organic
Good for nature
Good for you

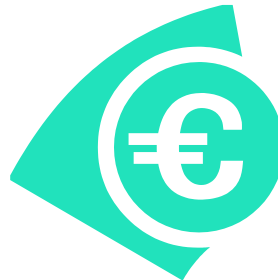


 **FSSC** 22000



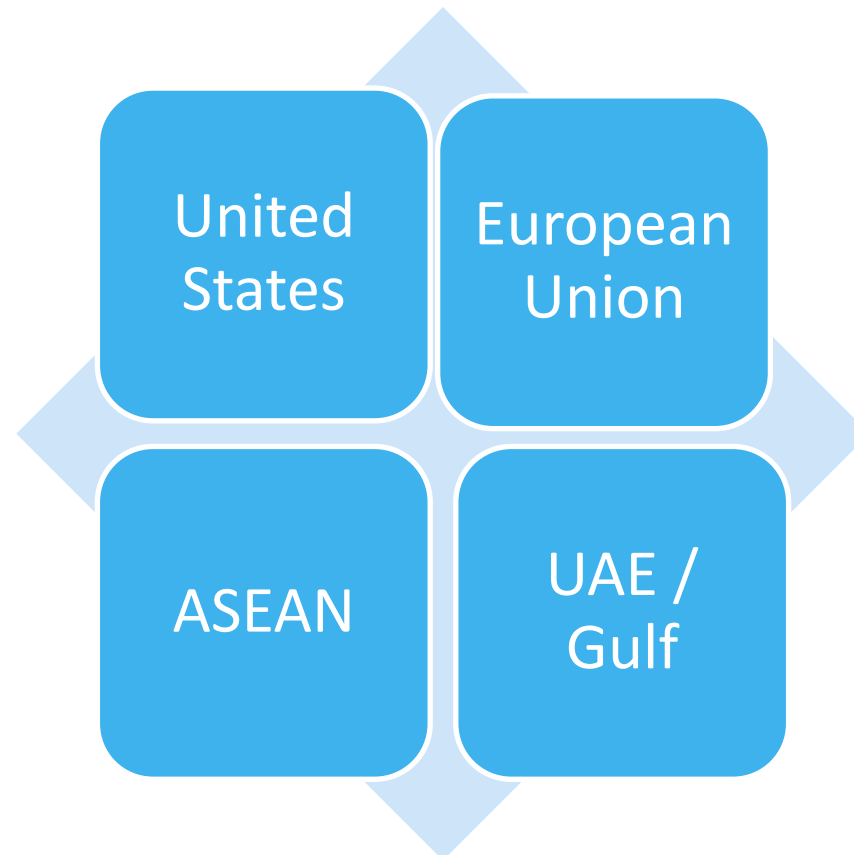
Why is ACCREDITATION important?

- Certification Bodies (CBs) are accredited by international accreditation bodies (IABs)
- **Accreditation** certifies competency, authority or credibility
- Accreditation ensures the CB's are competent to audit and certify third parties, behave ethically and employ suitable quality assurance .
- Accreditation ensures your certifications will be accepted in major markets / Major buyers (wholesale & retail)
- **ACCREDITATION = CREDIBLE QUALITY !**



Premium Markets, Food Safety and Certifications

-



TLR - Accredited Lab Testing



- ISO 17025 Accreditation
- Network of 26 labs
- EU and US require MRL / GFS Pesticide
- Organic Certification process requires MRL / GFS Pesticide testing
- Orchatoxin (Raisins)
- Moisture (nuts, fruit, cereals & Pulses)
- Food Safety Panel
 - Aflatoxin
 - E-coli
 - Salmonella
 - Bacterial Cerius

QUESTIONS





USAID
FROM THE AMERICAN PEOPLE

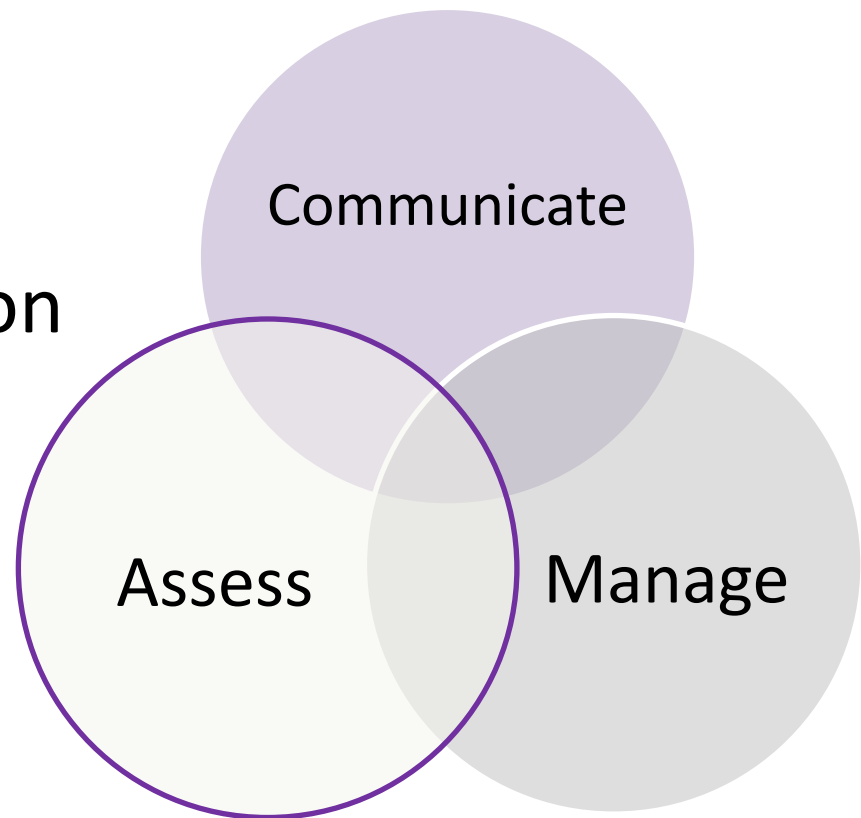
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Risk Communications



Risk Communications as part of Risk Analysis

- Risk Assessment
- Risk Management
- Risk Communication





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Risk Communications

Multiple roles in Risk Analysis process

- Developing “risk profile”
- Risk characterization
- Identify and weigh policy and decision alternatives
- Identify concerns beyond science
- Preparation of risk messages



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Elements of Risk Communications

- Share responsibilities – coalition
- Know your audiences
- Be a credible source



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Trust and Risk Perceptions

Major contributor to perceived risk

Public trust inverse to perceived risk

↓ public trust = ↑ perceived risk

↑ public trust = ↓ perceived risk

Perceptions about regulator inverse to perceived risk

Weak regulator = ↑ perceived risk

Strong regulator = ↓ perceived risk



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Trust

Influences method of risk communication used

Higher trust: less need for deliberative process

Receptive to outcome without knowing process

Lower trust: depends on reason for distrust

If impartiality, then deliberation increases fairness

Need to know or engage in process to accept outcome



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Risk Communications Action Planning

- Interactive process
- Exchange of
Information
Opinion
- Concerning
Risk
Risk-related factors
Risk perceptions
- *Throughout the Risk Analysis process*



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Risk Communications Action Planning

Among

Risk assessors

Risk managers

Consumers

Industry

Academics, and others

Includes

Risk assessment findings

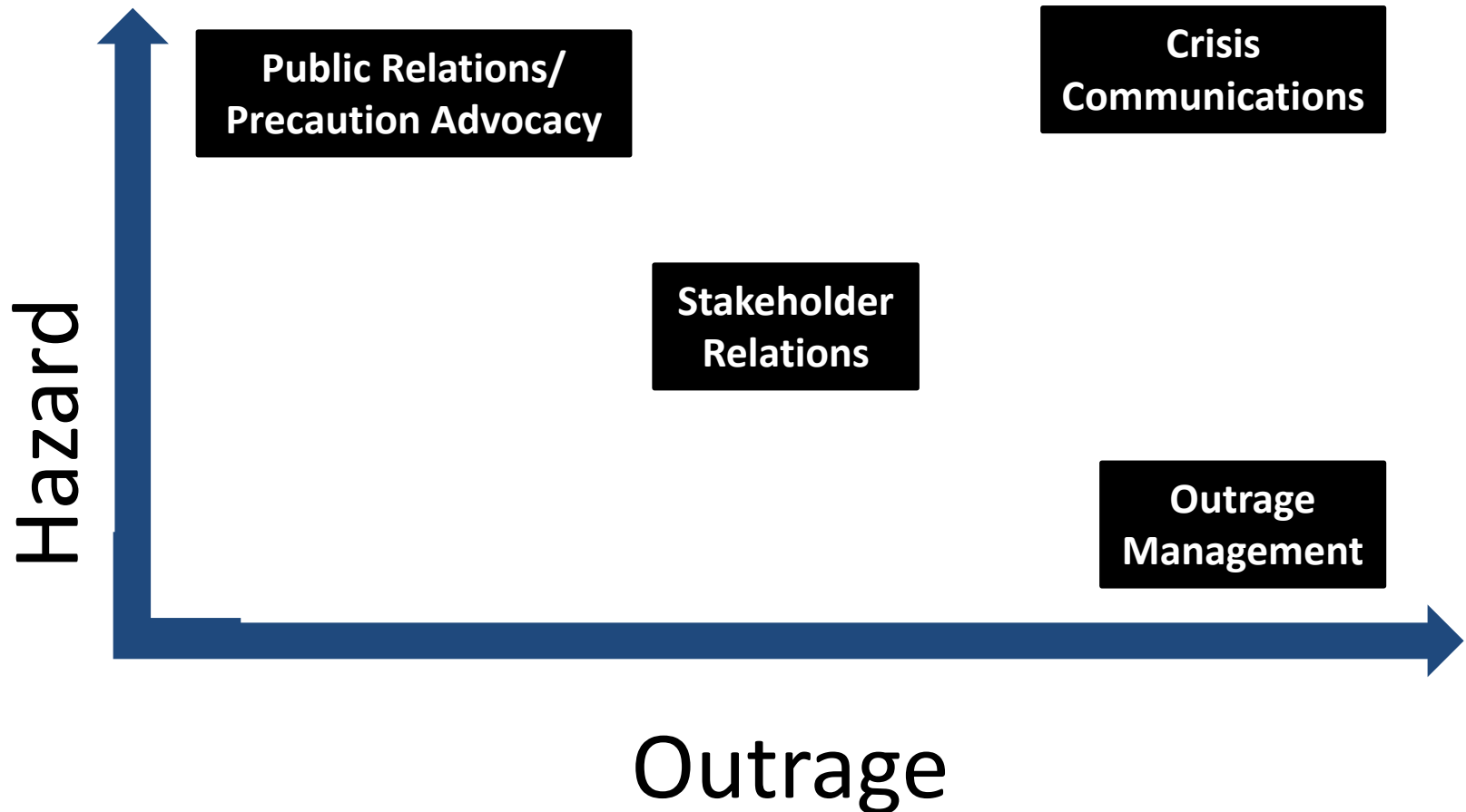
Basis of risk management decisions



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Four Kinds of Risk Communications





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Risk Communications Strategy

Systematic planning through

Background information

Preparation

Collaboration

Review and Evaluation

Awareness of Outrage factors

Familiarity and Frequency

Level of control

Morality and Ethics

Responsiveness of decision making



Pre-Harvest Mycotoxin Control

Ranajit Bandyopadhyay
IITA, Ibadan, Nigeria

Acknowledgement
Alejandro Ortega-Beltran
Themis Michailides, UC Davis, Kearney

- Highly toxic metabolite produced by the ubiquitous *Aspergillus Section Flavi*
- The fungus resides in soil and crop debris, infects crops and produces the toxin in the field and in stores
- Weak pathogen; injury and stress increase infection
- Survives for a long time; spread by wind and insects
- Climate change increasing incidence and severity of aflatoxins

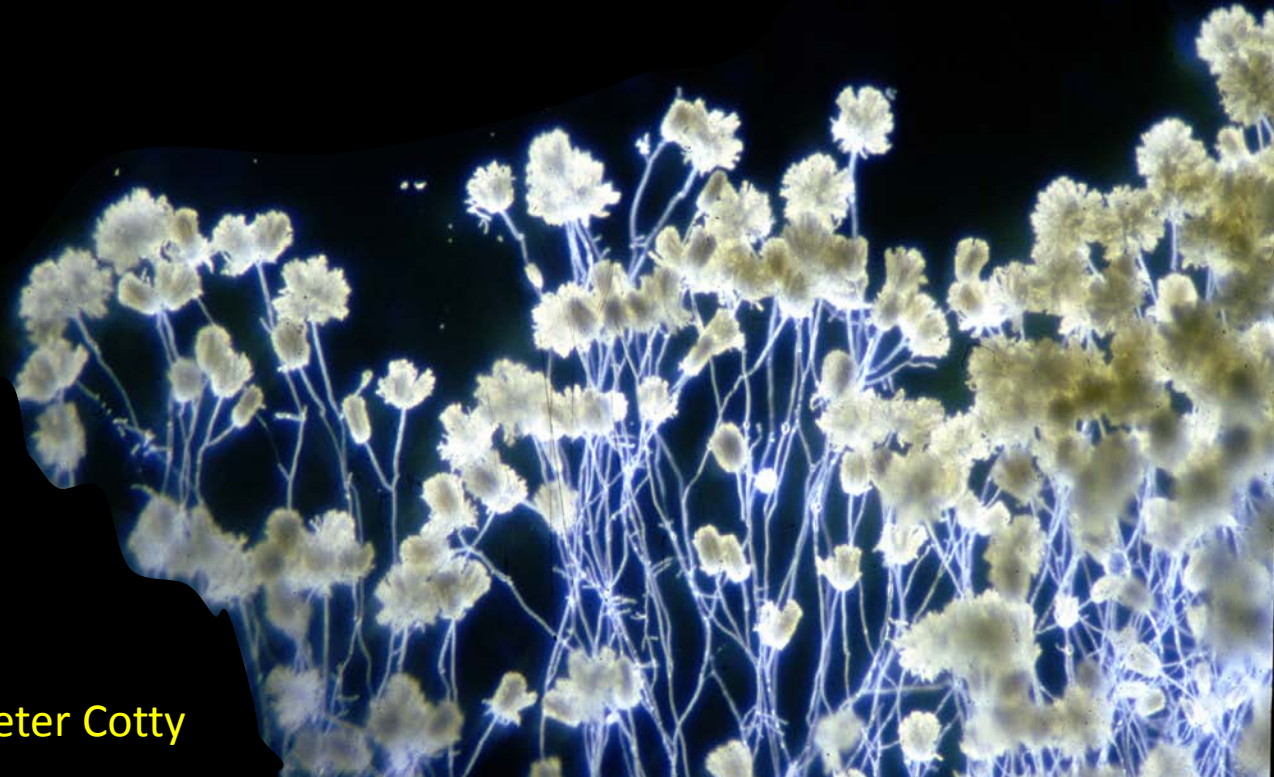
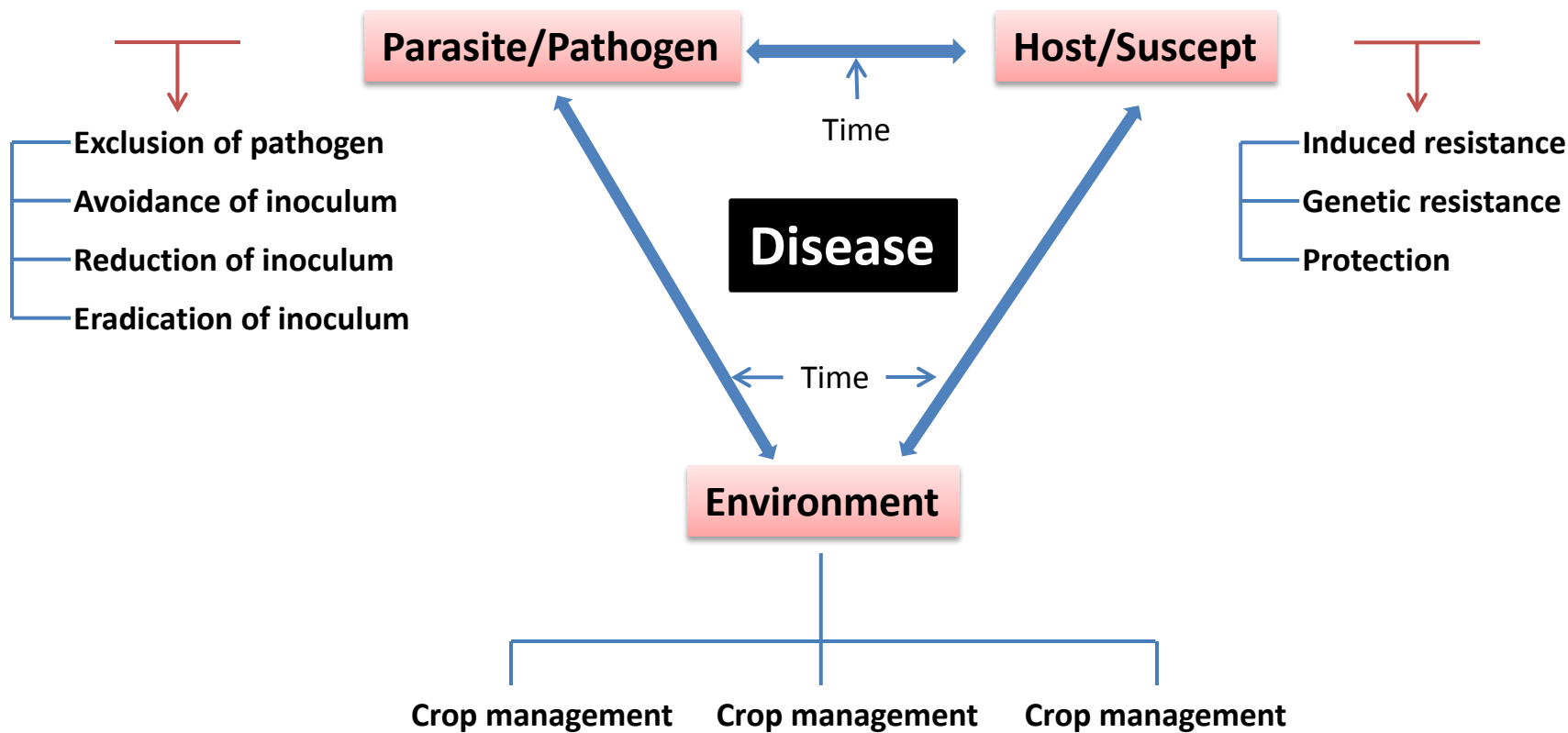
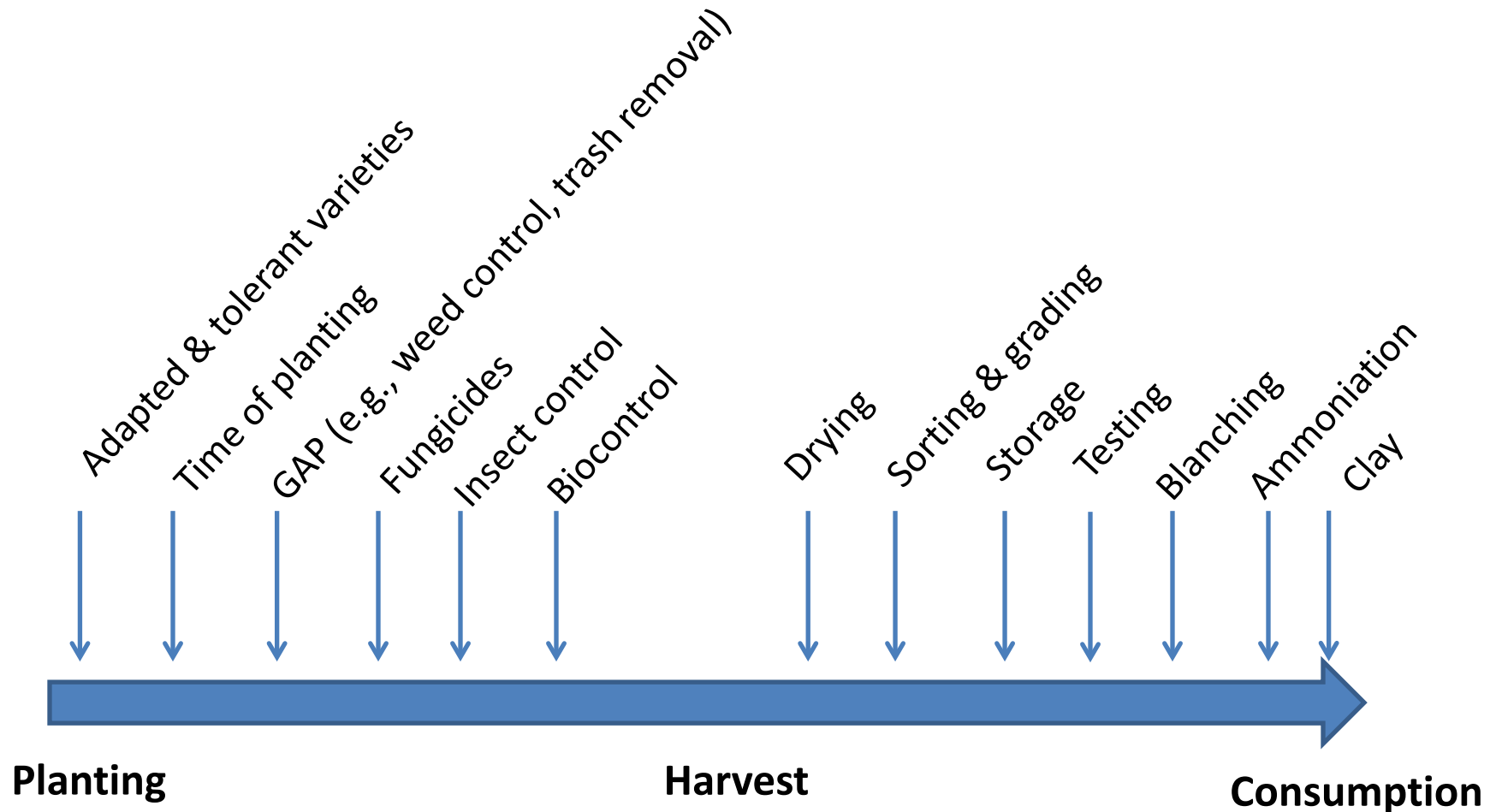


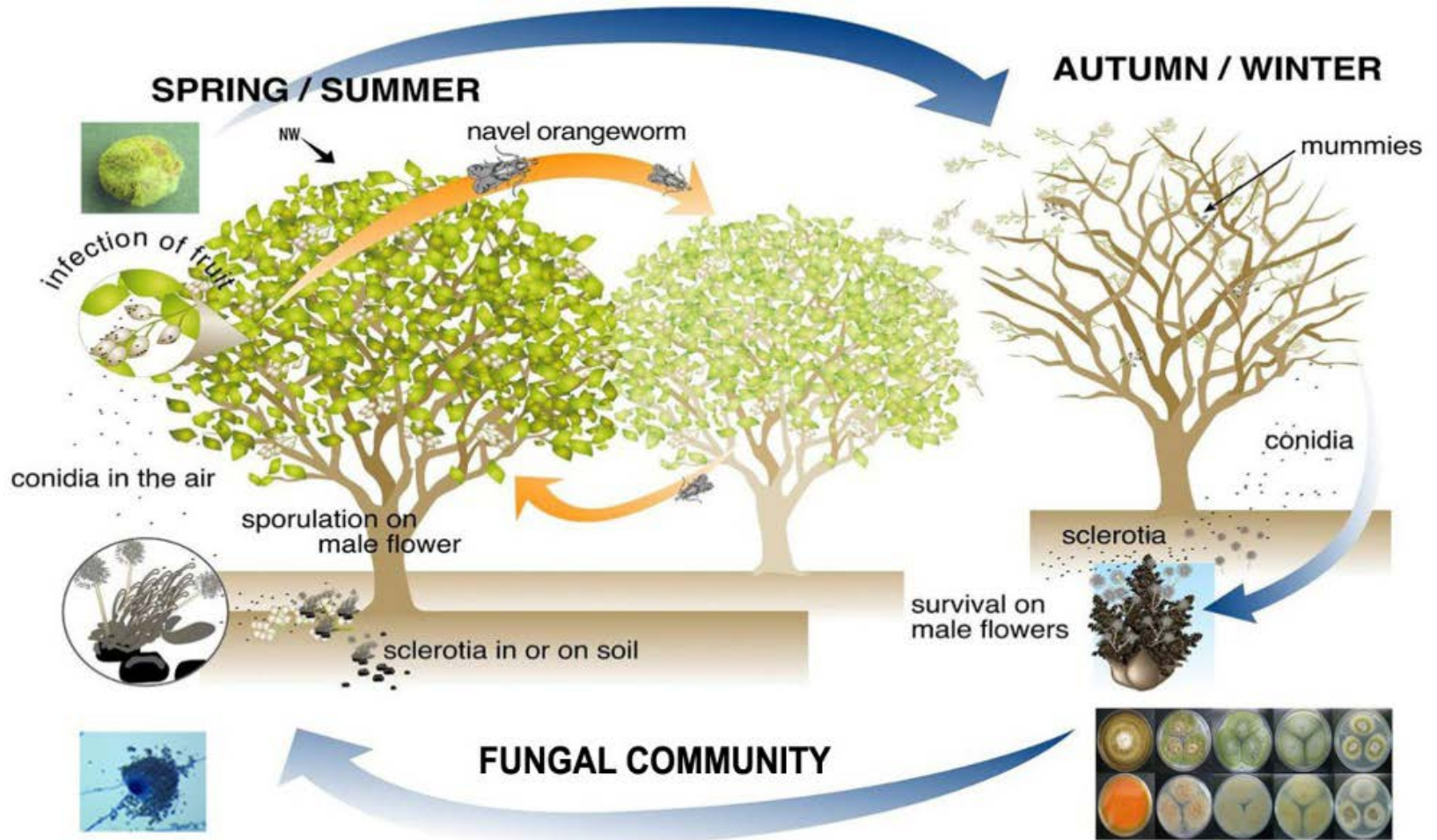
Photo: Peter Cotty



Multiple practices to Manage Mycotoxins



Aspergillus flavus life cycle in a pistachio orchard



Source: http://dx.doi.org/10.5772/45918_2



Primary source of aflatoxin contamination of pistachio kernels. These are frequently infested by both navel orange worm and aflatoxin-producing fungi

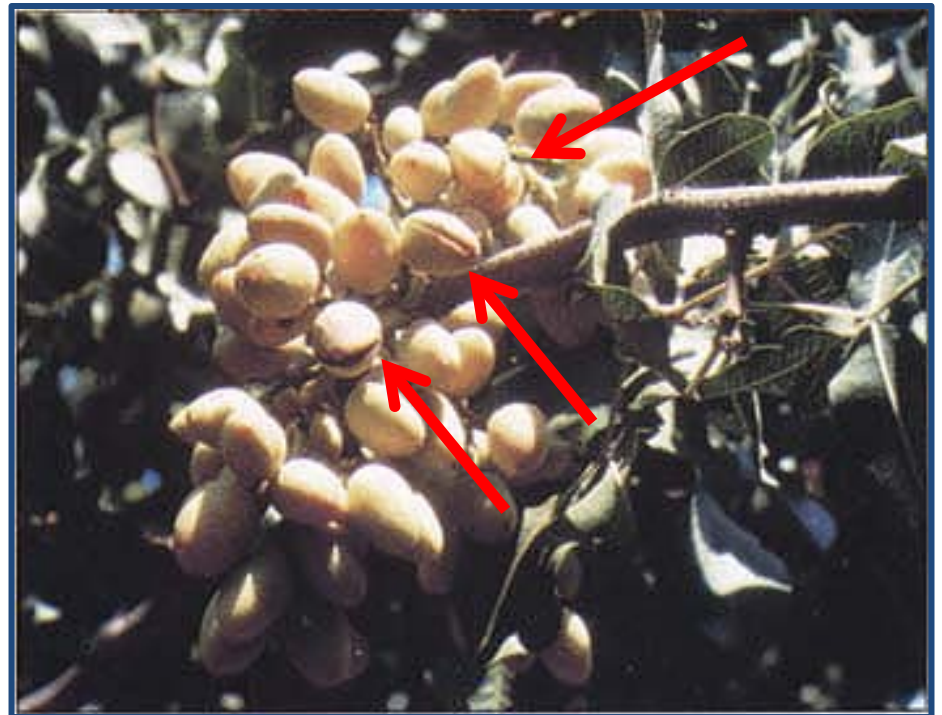


Photo credit: <http://californiaagriculture.ucanr.edu/>

Navel orange worm control using an insecticide (Belt®)



Treated, little to no insect damage = no aflatoxin contamination

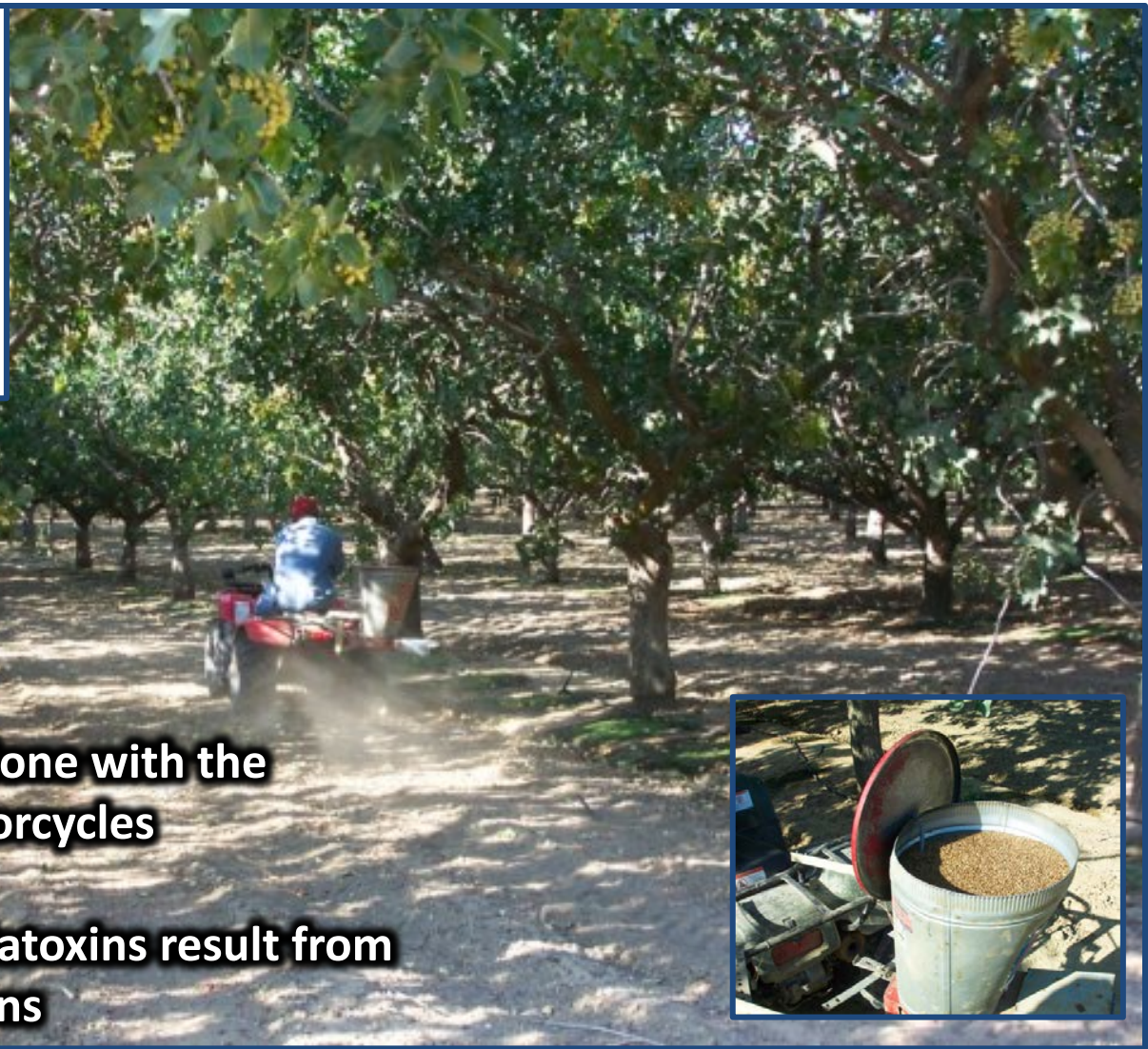
Untreated, severe insect damage = high aflatoxin contamination

Sprays should be done during the summer and after harvest



Decreasing the number of nuts left after harvest will reduce overwintering navel orange worm/*Aspergillus flavus* populations

Photo credit: <http://almonds.com>



Application are done with the aid of Quad Motorcycles

Over 50% less aflatoxins result from AF36's applications

Photo credit: <http://azcotton.org/>

- Application of biocontrol agents; use of AF36 results in at least 50% less aflatoxin concentrations in both pistachio and almond
- Conduct aggressive programs to control navel orange worm; sprayings should be conducted during spring and after harvest
- Reduce early split nuts (pistachio) by avoiding water stress during spring, applying dormancy-breaking chemicals before blooming, and avoiding cultivars prone to early splitting
- Elude late harvests
- Winter sanitation; remove nut mummies to reduce overwintering structures
- Dry nuts before storage
- Sort broken/damaged nuts and those with insect frass

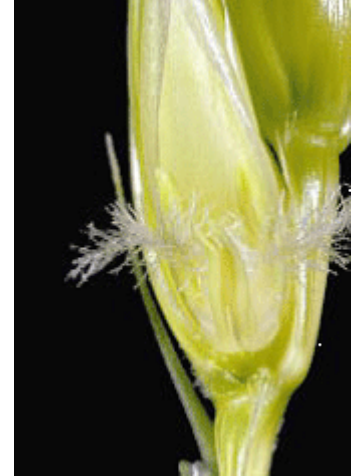
Wheat Ergot



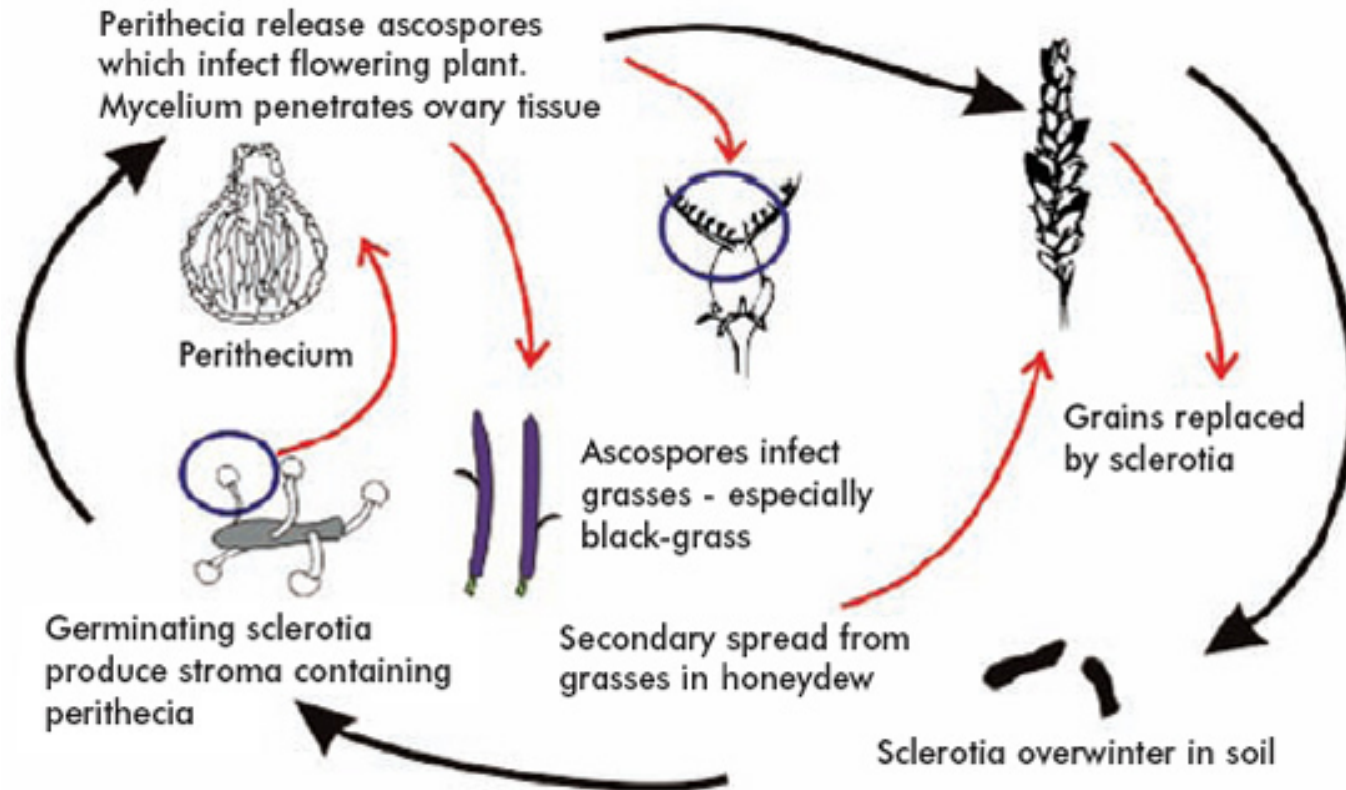
Source: www.apsnet.org

Wheat ergot

- Infection through stigma
- Pollination/fertilization shuts down infection
- Window of infection small
- Any factor that delay fertilization increases susceptibility (e.g., male sterility or low temperature or pollen wash)
- Duration of susceptibility is lengthened due to unevenness in flowering and the production of successive flushes of tillers
- Conidial inoculum from weeds such as Blackgrass infect maize flowers



Ergot Disease Cycle



Pre-harvest Management practices

Wheat Ergot

- Management practices that may influence ergot severity include crop rotation, management and nutrition, seeding practices, pesticide applications, nature of the crop (i.e. autumn vs. spring crop and self-pollinated vs. cross-pollinated host species).
- Benomyl spray at just before flower opening reduce infection. Not practical on a large scale.
- There is some degree of resistance in some cultivars
- Not very effective in controlling disease

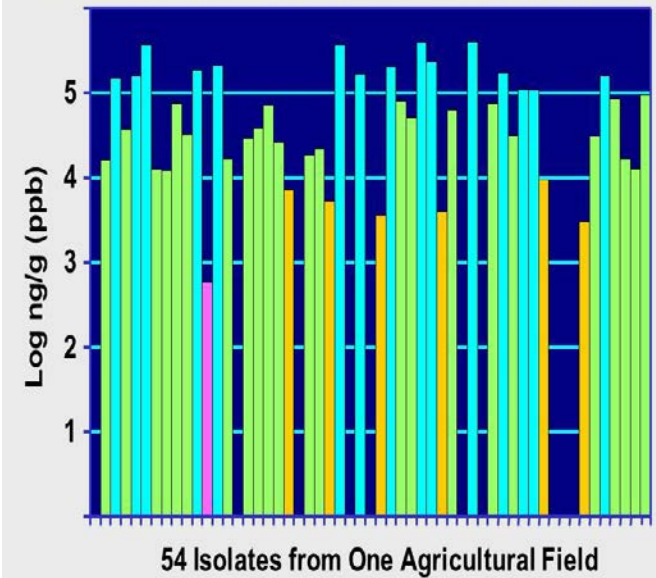
Wheat grains with ergot sclerotia



Source: University of Nebraska

- ▶ In nature, some strains produce a lot (toxigenic), and others no aflatoxin (atoxigenic)
- ▶ Atoxigenic strains are already present on the crop
- ▶ Increase the frequency of atoxigenic strains to competitively displace toxigenic strains to reduce aflatoxin contamination
- ▶ **Atoxigenic strains can be applied without increasing infection and without increasing the overall quantity of *A. flavus* on the crop or in the environment**

Aflatoxin Production by Fungal Isolates in Liquid Fermentation

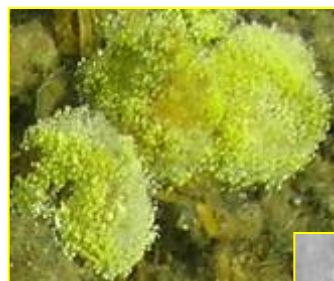


- ▶ Strains move from field to stores
- ▶ Multiple year & crop carry-over effect
- ▶ Use only native strains

How Does aflasafe Work?

Sporulation on moist soil

3-20 days

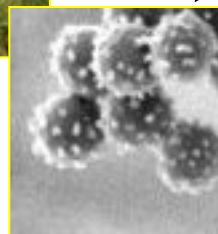


Soil colonization



Insects

Wind



Spores

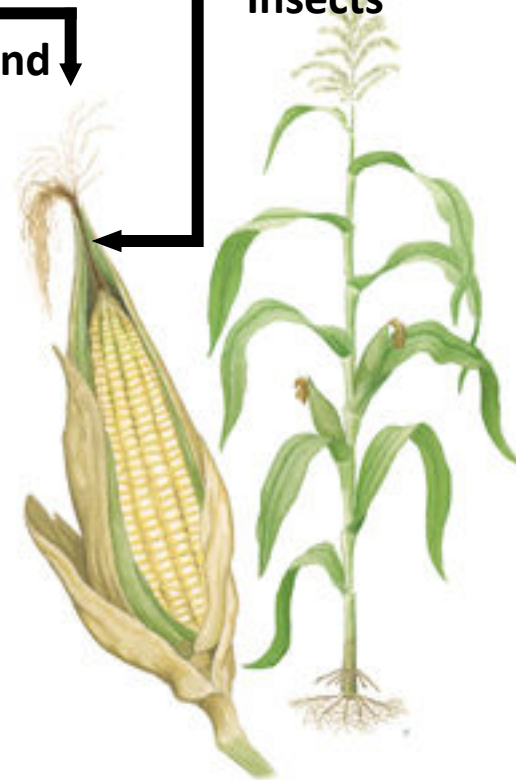
30-33 grains m⁻²



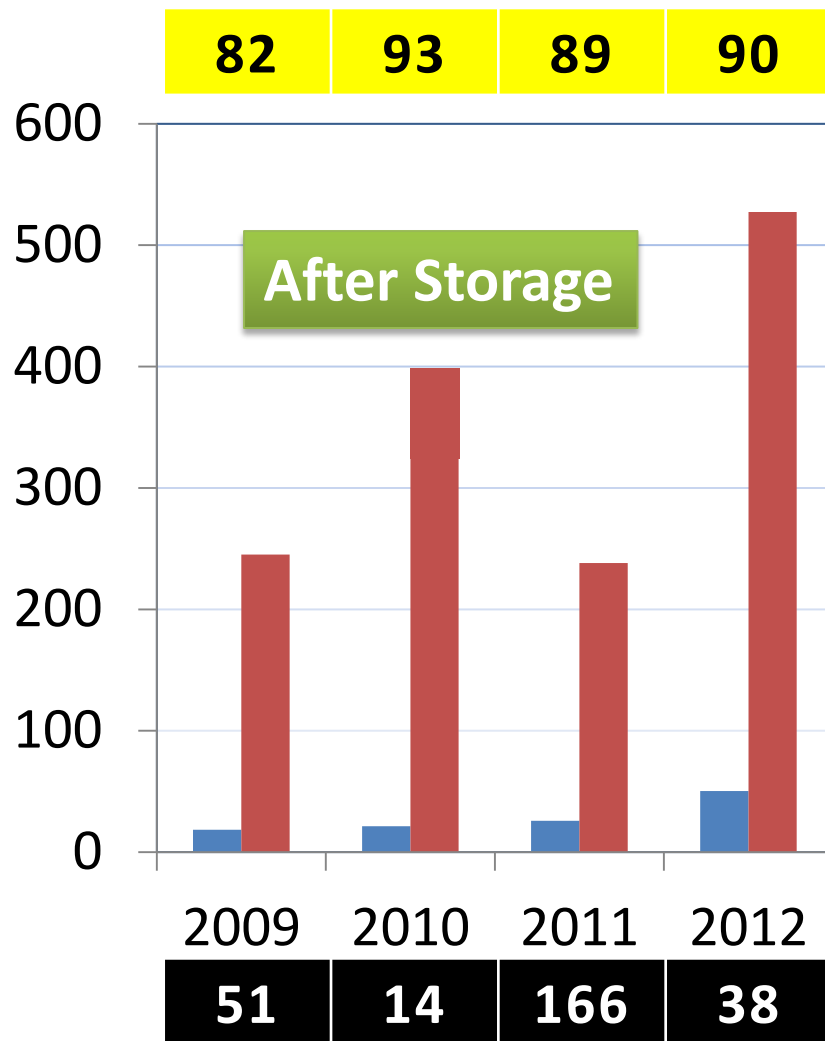
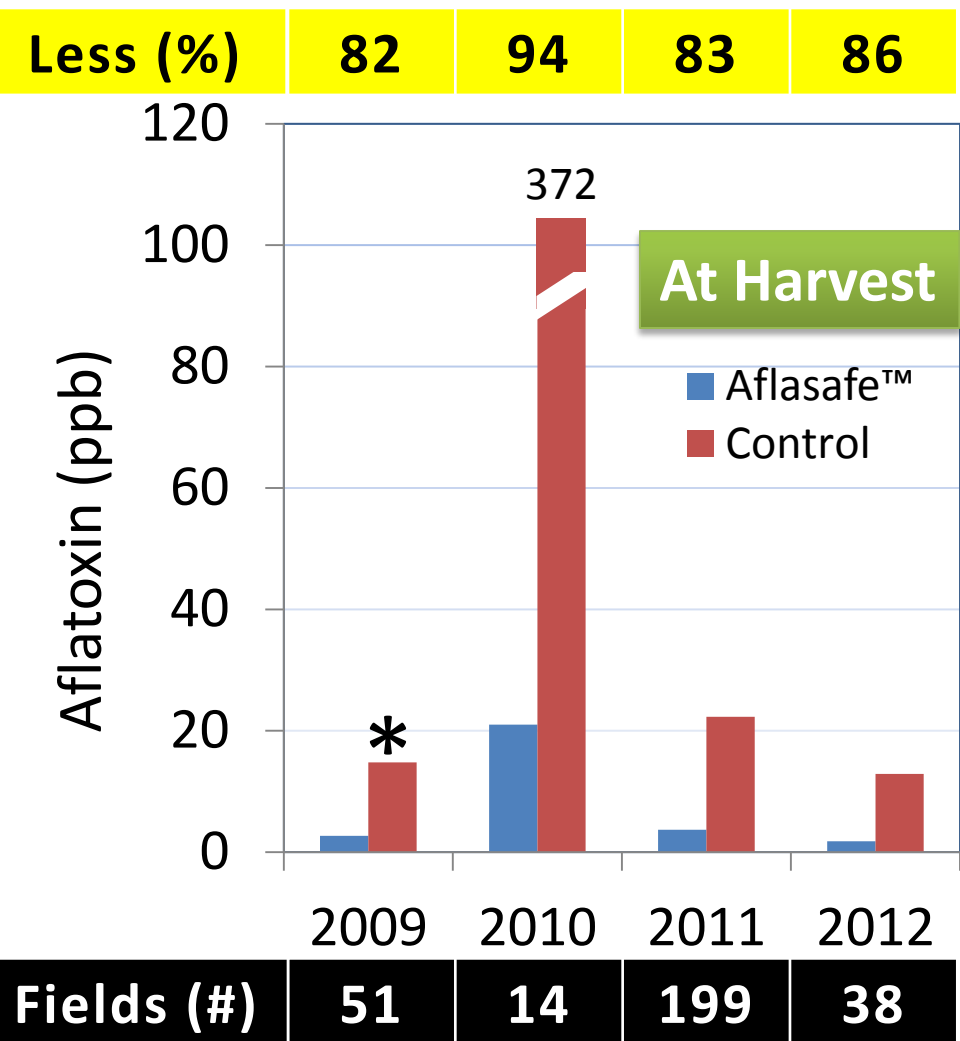
**Broadcast
@ 10 kg/ha 2-3 weeks
before flowering**



Aflasafe in 2.5 & 5 kg bags



Nigeria: Efficacy on Maize



*All means of aflasafe and control pairs significantly different; Student's t-test ($P < 0.05$)



Large-scale: capacity 5 tons/hour
Product cost: \$12 to \$18.75/ha



Parameters	2013/2014	2014/2015
Number of implementers	4	9
Total Aflasafe purchased (tons)	24	58.2
Number of farmers	1,015	3,271
Treated area (ha)	1,457	4,998
Maize aggregated for sale (tons)	2,031	7,220
Samples with <4 ppb AF (%)	99.0%	93%
Samples with <10 ppb AF (%)	99.5%	96%
samples with < 20 ppb AF (%)	99.5%	98%
Return on Investment (ROI)	210%	489%
Average sale price over market rate	13%	17%
Aflasafe maize kept for family	46%	20.3%

Grain lots meet international standards

Higher income

Better health

Smallholder farmers have safer crops, improved income and better health

Maize grown under center pivot in Galana-Kulaku, which is a part of 1 million acre Jubilee Food Security project of the Kenyan Govt.

Maize crop being treated with Aflasafe KE01 in Galana

- Managed by the National Irrigation Board (NIB)
- Highly productive area but aflatoxin-prone
- Maize frequently rejected as >50% strains in soil are highly toxic

- 238 tons aflasafe ordered (8.1 tons airlifted for emergency treatment) from IITA in Nigeria
- The entire crop of 200 ha treated with aflasafe
- **Harvested grains had <4 ppb aflatoxins (meets strict European limit) in spite of delayed harvest**



Aflasafe KE01 in the Aflasafe factory in IITA-Nigeria ready for shipment to Kenya



Principles of Food Safety & Quality

**Presented at the USAID Workshop on Mycotoxins
Delhi, India, March 13-15, 2016**

**John D. Floros, PhD
Professor of Food Science & Engineering
Dean of Agriculture & Director of KSRE
Kansas State University
Manhattan, KS 66506, USA**



Topics of Discussion

- **Food Safety & Quality Defined**
- **Types of Food Hazards**
- **Impact of Foodborne Illnesses**
- **Surveillance & Regulation**
- **Food Safety & Traceability**
- **Shared Responsibility**



Food Safety Defined

Food Safety is a process/system that makes food safe to eat and free of disease causing agents:

- Sufficient number of infectious agents (e.g. Bacteria, Fungi, Viruses, Parasites)
- Toxins (e.g. mycotoxins)
- Toxic chemicals
- Foreign objects



Food Quality Defined

Food Quality is a process/system that makes a food desirable to eat with regards to taste, flavor, smell, color, and texture; Unacceptable food quality can be judged by:

- Inappropriate color
- Wrong texture
- Bad odor/smell
- Questionable temperature history



Types of Food Hazards

- **Biological**: bacteria, fungi, viruses, parasites
- **Chemical**: heavy metals, natural toxins, sanitizers, pesticides, antibiotics
- **Physical**: bones, rocks, metal, glass



Biological (= Living Organisms) ***Hazards in Food***

- In Poultry & Eggs, and Meat & Dairy Products:
 - *Salmonella* bacteria
 - *Listeria Monosytogenes*
- On Fruits & Vegetables:
 - *E. coli* bacteria
 - *Cyclospora* parasite
 - Hepatitis A virus
- Grains, Nuts and Other Dried Foods
 - Fungi (Molds & Mycotoxins)



Prevention of Biological Hazard to Foods

Cooking at High Temperature kills microbes

- > 73° C for poultry and eggs
- > 68° C for ground beef
- > 74° C for milk & dairy
- > 95° C for plant based foods

Microbes can't grow at:

- Low temperatures of < 5°C
- Cool foods quickly from 60°C to 5° C



Chemical Hazards in Food

Chemical hazard: a toxic substance that is produced naturally or added intentionally or un-intentionally

- Naturally-occurring:
 - Natural Toxins (mycotoxins, botulinum toxins)
- Added intentionally:
 - Antibiotics, Preservatives
- Added non-intentionally:
 - Cleaning Agents, Pesticide Residues



Physical Hazards in Food

Physical hazard: a hard foreign object that can cause illness or injury

- Inherent to the food or ingredient
 - Bone fragments, feathers
- Contaminant during processing
 - Stones, rocks, dirt, fingernails, glass, metal



Foodborne Illness Defined

An illnesses caused by agents that enter the body through the ingestion of food.

- Every person is at risk of foodborne illness.
- May be serious for very young, very old, people with long term illness
- Reaction may occur in a few hours, up to several days after exposure, or it may take several years (stunting)

Symptoms

- Abdominal cramps, headache, vomiting, diarrhea (may be bloody), fever, death



The Impact of Foodborne Illnesses

- The global incidence of foodborne disease is difficult to estimate
- Up to 30% of the population annually
- 2.1 million people died from diarrheal diseases (2001)
 - Food & Water Contamination



The Impact of Foodborne Illnesses

In the US annually:

- 76 million cases of foodborne diseases
 - 325,000 hospitalizations
 - 5,000 deaths

In China (1994) Salmonella Outbreak estimated
224,000 persons



Surveillance/Regulation

- Surveillance
 - CDC
 - FoodNet and PulseNet
- Regulation
 - FDA
 - Domestic and imported food
 - USDA FSIS
 - Meat, eggs, poultry



Hazard Analysis Critical Control Point (HACCP)

The purpose of HACCP is to ensure production of safe food

- The goal of HACCP is to prevent and/or minimize risks associated with biological, chemical, and physical hazards to acceptable levels
- It is based on **PREVENTION** rather than detection of hazards
- Pioneered in the 1960's: first used for the space program (Pillsbury & NASA)



The Steps involved in HACCP

1. Identify hazards
2. Determine Critical Control Points (CCPs)
3. Determine safety limits for CCPs
4. Monitor CCPs
5. Take corrective actions
6. Record data
7. Verify that the system is working

HAZARD RISK ASSESSMENT MATRIX

Frequency of Occurrence	Hazard Categories			
	1 Catastrophic	2 Critical	3 Serious	4 Minor
(A) Frequent	1A	2A	3A	4A
(B) Probable	1B	2B	3B	4B
(C) Occasional	1C	2C	3C	4C
(D) Remote	1D	2D	3D	4D
(E) Improbable	1E	2E	3E	4E



Unacceptable



High



Medium



Low

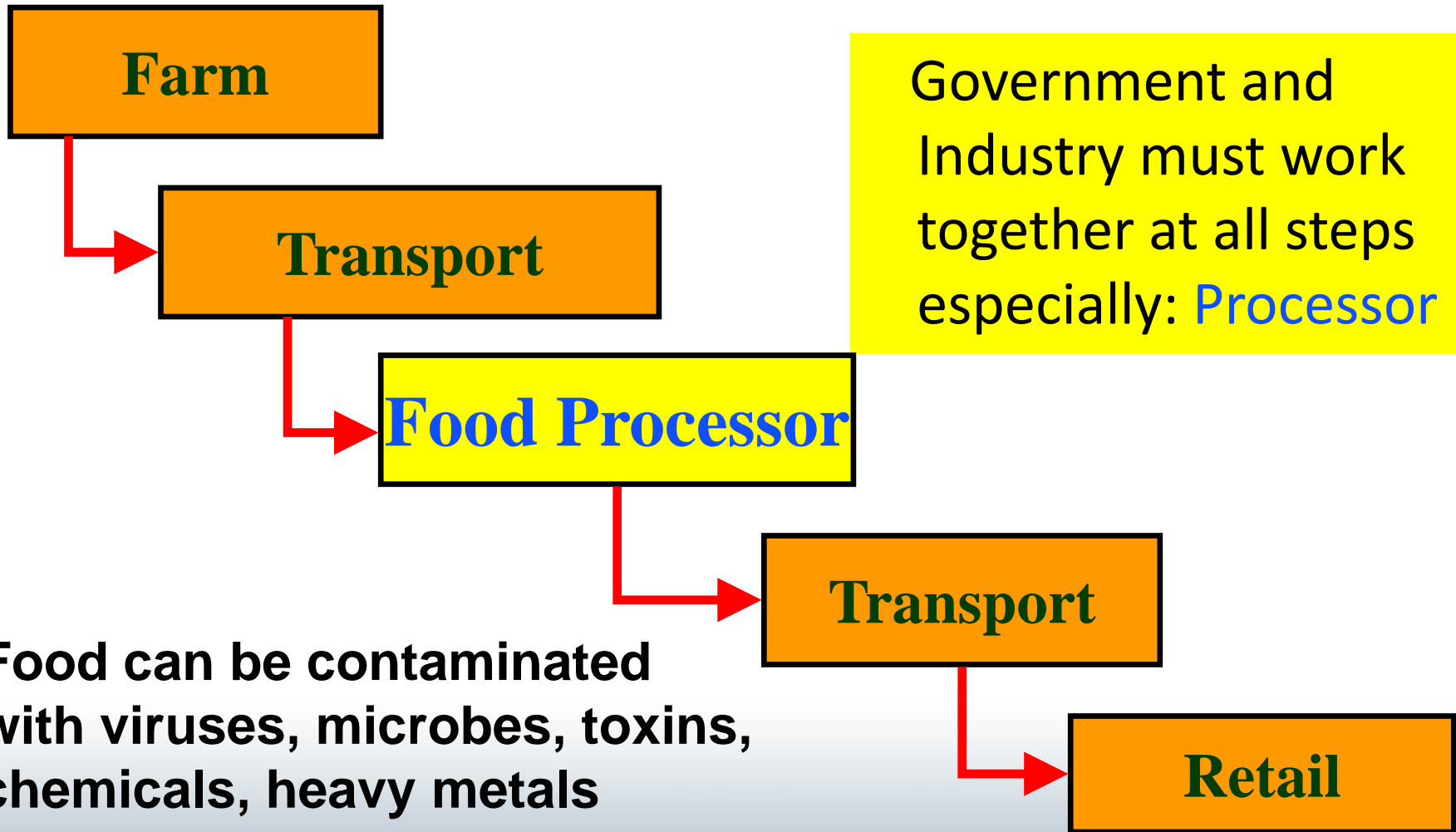


National Grain and Feed Association

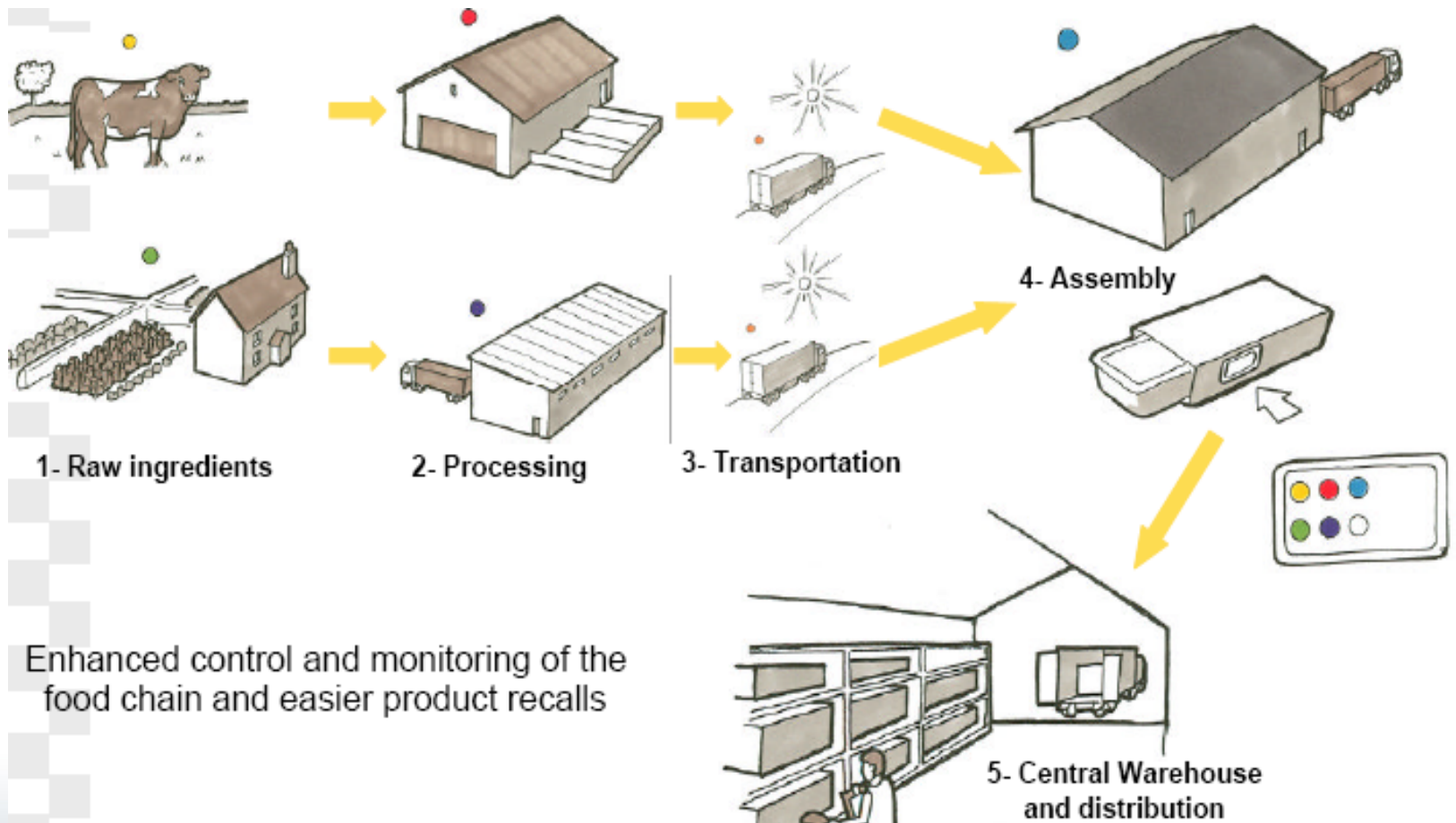
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Issues Impacting Food Safety



Food Safety and Traceability



SAFE FOOD FOR ALL

SHARED RESPONSIBILITY

Food Legislation and Enforcement	Educated and Knowledgeable Public	Good Practices by Primary Producers and Distributors
Advice for Industry/Trade	Discriminating and Selective Consumers	Quality Assurance and Control of Processed Food
Consumer Education	Safe Food Practices in the Home	Appropriate Processes and Technology
Information Gathering and Research	Community Participation	Trained Managers and Food Handler
Provision of Health-Related Services	Active Consumer Groups	Informative Labeling and Consumer Education
GOVERNMENT	CONSUMER	INDUSTRY/TRADE

WHO Leadership for International Consensus on Food Safety Issues, Policies, and Actions



Additional Resources

- Centers for Disease Control and Prevention
 - <http://www.cdc.gov/foodsafety/>
- U.S. Department of Agriculture
 - <http://www.foodsafety.gov>
 - <http://fnic.nal.usda.gov/about-fnic>
- Food and Drug Administration (FDA)
 - <http://www.fda.gov/Food/>



Questions?

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Where Do We Go Now?

John Leslie

John Floros

Unofficial Conference Goal

- Begin thinking and planning about where to go based on the data collected so far
- This project was an assessment of contamination, and there is little in the published literature on these toxins in Central Asia
- We needed to write an action plan. We haven't, but we have been setting priorities needed to establish one

Efforts for This Week and This Month

- Synthesize nominal group results and return to Mac.
- Develop teams in Afghanistan to make decisions and help design implementation strategies.
- Identify appropriate international partners
- Help the effort grow to a food safety effort that is broader than just mycotoxins

What Are Some Problems?

- Confidence in Data Collection and Results
 - Training & Education
 - Facilities
 - Communication
 - Politics
-
- Action Items and Next Steps

Training/Education

- Short courses
 - Where?
 - On what?
- Long term
 - Degree granting?
 - Focus on no more than a few value chains?
 - Other than agriculture?
 - Network building?

Facilities

- Buildings
- Infrastructure
- Maintenance
- Security
- Equipment
- Supplies

Communication

- Who is taking the lead?
- Between governments in the region
- Between people in different parts of the Government in Afghanistan
- Between Donors and the government
- Between donors
- Between the government and
 - Public (consumers, etc)
 - Industry
 - Farmers

Politics

- Corruption
- When “no” is an unacceptable answer
- When something else comes first
- When the government changes
- Who gets credit
- Who makes decisions

A Few of Our Ideas

- Separate research (information gathering) from regulation
- Involve university staff and students
- Collect baseline data
- Routine back up for controversial results
- Become part of the international conversation on mycotoxins
- Take advantage of your uniqueness
- Steal good ideas shamelessly (with credit)

A Few Examples of Actions

- Short Term
 - Train people in Food Safety Principles
 - Clean up the Ergot
 - Identify Alternative Markets for products
- Long Term
 - Develop Communication Strategies for ...
 - Train MS & PhD level scientists
 - Develop Import Standards and Regulations

Round 1

- Short term
 - National Survey of mycotoxins
 - Food Safety Laws
 - Form Commodity Associations
 - Infrastructure development
 - Identify ministry roles in mycotoxins
- Long term
 - Regional mycotoxin labs
 - Organize a Food Safety Authority
 - Government certification of private labs
 - Technical help for the private sector
 - Promotion of healthy food

Round 2

- Short Term
 - Create private sector labs accredited by the government
 - Training courses for farmers
 - Create inter-ministerial working groups
 - Find Money - Donors
 - Coordinate Ministries and ACCI
 - Establish mycotoxin regulations
- Long Term
 - Dietary Guidelines
 - Good Pre- and Post-Harvest Practices
 - Find money – Government
 - HACCP studies of each commodity and forest products
 - Develop mitigation strategies for mycotoxins

Now

- Initial Delhi workshop to define problems and get Agriculture, Health and Commerce/Trade ministries to begin talking with one another about food safety problems.
- Begin conversations with neighboring countries to determine areas of mutual interest. Determine if any actions considered below are more appropriate at regional than at Afghanistan only level. Goal is collaboration not antagonism.
- Identify appropriate university staff to involve in various programs.
- Develop a long-term training program to build capacity in mycotoxins and the related, health, agricultural, trade and regulatory areas. Should the focus be to establish a “center of excellence” somewhere in the region and staff it with individuals from multiple countries? How broad and how large does the cohort of trainees need to be. Develop training program in such a manner as to enable strong networking amongst the trainees.
- Continue collecting baseline data on toxin levels associated with various weather, location and cropping conditions.
- Develop alternative process for shelling walnuts that does not require the shells to be wet before cracking.
- Develop capacity to screen milk for aflatoxin M₁.
- Develop in-country protocol for validation of high-level contamination detection.
- Training in grain cleaning.
- Develop regulations/legislation defining mycotoxin limits, sampling protocols, and acceptance/rejection of imported materials. Goal is to apply legislation to large imports, although the law/regulation could be written to be more widely applicable.
- Establish a 2nd in-country screening process.
- Establish a formal link with a European Lab, *e.g.*, BOKU or ISPA, for back-up work with unusual or significant samples.
- Send appropriate staff (MAIL, others?) to one week Mycotoxin training workshop at ISPA.
- Study group to assess risks posed by chronic exposure to subacute levels of multiple mycotoxins. Identify potential remediation steps including clays, yeasts and other probiotics potentially incorporable into human foods. Determine feasibility of incorporating screening of mycotoxin biomarkers in humans (from urine) with study already planned to evaluate micronutrients. Identify tolerable daily intakes given typical Afghan diets. Risk communication strategies.
- Study group to evaluate post-harvest treatment and utilization options for contaminated materials. Ammoniation, ozonation, chlorine dioxide, sorting and dilution/blending as “treatments”. Brewing, biofuels, fermentation feedstock, food/feed processing as utilization options. Determine when material must be destroyed and the conditions under which that should happen. Consider gender/societal implementation issues. Focus on science of acceptability of treated products which might not be allowed elsewhere for economic reasons. Risk communication strategies.
- Study group to evaluate value/production chains for tree nuts and raisins. Define current Afghanistan practices and gender/sociological/technical barriers to changing them. Identify technology available for implementation – crop varieties, fungicides, accelerated drying techniques, hermetic storage, *etc.* – and associated timeline(s). Identify barriers to entering alternative markets – mycotoxins, pesticide residues, shipping, time of year demand, *etc.*, and develop strategies to overcome them. Risk communication strategies.

- Develop short-term training for in-country staff and “extension” personnel.
- Develop small external consulting team to advise mission on food safety and mycotoxins – quarterly video meetings, or more frequent if reason.

Medium term

- Delhi II conference.
- Continue collecting baseline data on toxin levels associated with various weather, location and cropping conditions.
- Develop educational modules for the general public in Afghanistan regarding dangers of mycotoxins and things that can be done to lessen their health and trade impacts.
- Send appropriate staff (MAIL, others?) to one week *Fusarium* Laboratory Workshop at K-State (June).
- Begin biomonitoring to assess human exposure to mycotoxins. If possible, correlate analysis with samples from foods that were consumed.
- Study group to evaluate Good Agricultural, Drying and Storage Practices in wheat. Define current Afghanistan practices and gender/sociological/technical barriers to changing them. Identify technology available for implementation – crop varieties, rotation, fungicides, accelerated drying techniques, hermetic storage, *etc.* – and timeline for doing so. Design local wheat breeding program needs in terms of staff and capacity.
- Evaluate wheat milling and storage protocols with two goals: reducing aflatoxin contamination (which occurs primarily in storage) and determining which milled fractions are enriched for mycotoxin contamination. Focus on conditions in Asiab mills and in commercial mills that differ.
- Evaluate small business model for moving drying, sorting & cleaning, with sales of hermetic storage bags.
- Assess personnel and institutional capacity needed to conduct a wheat breeding program to screen for resistance to diseases and mycotoxin contamination. Identify fungal species, their frequency and their mycotoxin production capacity in wheat fields in Afghanistan and countries from whom wheat is imported.
- Develop local technology for an AflaSafe program for raisins and tree nuts. Assess biodiversity of fungi present in these products with goal of identifying aflatoxin producers and non-producers.
- Broaden mycotoxin testing in country, based on capacity already developed. Identify crops/products to be tested and when/where it is to be done. Identify sustainable source of funding. If providing certification, keep process corruption free. Decide if mycotoxin screening is optional, or mandatory for exports (brand protection) and imports, and develop appropriate policies.
- Implement actions developed by post-harvest treatment/utilization study group.

Longer term

- Delhi III conference.
- Continue collecting baseline data on toxin levels associated with various weather, location and cropping conditions.
- Field testing of AflaSafe program.
- Implement local wheat breeding program.
- Implement reliable mycotoxin screening program.

Complete Nominal Group Process and Results

19 Nominal Group Technique discussion guidelines

Groups will be given questions by the conference organizers. The Discussion of each question will follow a structured process outlined below. The process is designed to generate the largest number of ideas and to provide equal opportunity for input from all participants. There are no “right” or “wrong” answers to the questions. At the end of the discussion participants in each group will rank the answers generated. If time permits, then there may be a general discussion of the results from different groups at the end of the session.

Each group has a facilitator, who will run the session, and a reporter, who writes ideas on a flip chart. When the groups meet for the first time take a few minutes to go around the room and introduce yourself to the other participants, and indicate why you are interested in the topics of mycotoxins and food safety.

Stage 1 (8-10 minutes). Silent generation of ideas.

The question to be considered and its number is written on the flip chart. Each individual receives a set of three white 3×5 cards. On the 3×5 cards each individual should write the number of the question, the name of their group, and the ideas/answers that come to mind as potential responses to the question. Put one idea/answer per card (more cards available if needed). Participants should **not** discuss their ideas or talk with other participants in the group during this time.

Stage 2 (10-12 minutes). Sharing ideas.

Participants go around the group and share **one** of their ideas/answers. The reporter goes first and writes down each response verbatim as a word or phrase on the flip chart. The facilitator goes last. There is no debate of ideas at this time. If a participant has a new idea while listening to the others in the group they may add it to their list. Adding responses to the list continues by going around the group as long as necessary until there are no more ideas to be presented. There is no expectation that each member of the group will provide the same number of responses, and individuals can continue to add items to the list until their list is exhausted. When all participants have passed in a round of idea sharing, then this stage comes to an end. Cards with ideas written on them should be turned in to the reporter. Include the name of the group on each card.

Stage 3 (30 minutes). Idea explanation.

This discussion is moderated by the facilitator. The goal is to make sure that the ideas are clearly understood by all members of the group. One way to conduct the discussion is to go around the group with each member raising a question at a time on a particular idea. Alternatively, the facilitator may move through the list on the flip chart in a systematic manner that ensures every response is discussed. All participants in the group can contribute to the discussion of an idea, and the reporter may indicate modifications or clarifications on the flip chart. Avoiding judgement and criticism is important at this time and the process should be emotionally neutral. The facilitator should ensure that everyone participates in the discussion in an equal manner. Ideas may be grouped if the participants who suggested them agree that they are the same. It is important not to spend too much time on any one topic and to ensure that everyone who wants to

comment on a response is allowed to do so. The group may suggest new responses for the list and combine ideas presented by different people.

Stage 4 (10 minutes). Voting and Ranking.

At the end of the period, each participant is given a colored 3×5 card. Write the question number and the group name on the card. The five most important answers for the question are ranked on the card with the most important idea being given a “5”. The second choice response receives a “4”, and the next a “3” and so on. Each individual must rank five responses on their list. The 3×5 cards are turned into the reporter who tallies the phrases selected by each individual on the flip chart. The flip chart paper and the 3×5 cards with the rankings are turned into the conference organizers for further analysis. The reporter and chair may be asked to present their group’s conclusions at the wrap-up session at the end of the day, if time permits.

20 Nominal Group Questions and Responses

Question T-1

Identify Capacity Building required for a sustainable mycotoxin surveillance program in Afghanistan.

	City		Lake		Mountain		Ocean		Valley		Response
	^x #	^y S	#	S	#	S	#	S	#	S	
1	●	●	5	16	2	10	3	10			Increased public awareness
2	●	●	3	8	●	●			3	8	Data repository (toxins and crops) for management purposes
3	4	13	4	7			1	3			Undergraduate & graduate training in food safety
4					1	5	4	10	3	7	Appropriate labs with enough space and equipment
5			●	●	3	8	3	6			Sufficient trained staff (lab & inspectors)
6	●	●					2	6	2	6	Collaboration with international academic institutions (long term)
7					1	4	●	●	1	3	Training course for traders, food processors, exporters & importers
8	5	21			5	15					Adequate sampling protocols/strategies
9					5	19	3	8			Established upper limits for mycotoxin contamination
10	5	21							3	6	Develop surveillance system
11			6	27					1	3	Develop a food safety authority
12					4	11			3	7	Accredited labs
13			2	3			4	18			Government funding commitment
14			6	22			●	●			Labs to check for quarantine issues – certified
15			6	22			●	●			Fee-based screening labs – certified
16					3	5	1	1			Multiple detection methods (robust detection)
17	3	8					1	2			Good Agricultural Practices (pre-harvest)
18	3	8					●	●			Good Agricultural Practices –Post-Harvest
19							1	3	1	5	Interministerial and private sector task force
20					1	4			1	1	Training for farmers
21			2	3			●	●			Private sector involvement
22	5	18									Sample selection protocols
23							5	12			University/academic research
24							4	14			Train the trainers program
25			4	9							Certification program for sampling, analysis, surveillance & report preparation
26	4	8									Train lab staff in proper data collection
27							3	13			Funds from non-governmental sources

	City		Lake		Mountain		Ocean		Valley		Response
	^x #	^y S	#	S	#	S	#	S	#	S	
28									3	11	Technical training throughout the value chain
29									3	11	Technical training for laboratory staff
30									3	9	National guideline/framework
31	3	5									Train staff on proper data analysis
32									2	10	Short/Long term training for govt. employees
33							2	8			Labs in provinces across the country
34	2	3									Train staff on proper data entry
35	2	3									SOP for sampling
36									1	5	Institutional capacity development
37					1	1					Proficiency tests for laboratory staff
38	•	•									Train staff in equipment use
39	•	•									Train staff in data validation
40	•	•									Trend analyses
41			•	•							Management capacity to prepare plans and analyze results
42					•	•					Weather data from growing areas
43					•	•					Distribution of results from current survey
44							•	•			Central lab in Kabul
45							•	•			NGO and CSO involvement
46							•	•			Participation from farmer's cooperatives
47							•	•			Enabling environment for the work
48							•	•			Regional (international) collaboration
49									•	•	Well trained agriculture faculty
50									•	•	Practical application of theoretical knowledge and skills

Question T-2

Identify data that should be collected to enable decisions regarding mycotoxin contamination to be made in Afghanistan.

	City		Lake		Mountain		Response
	^x #	^y S	#	S	#	S	
1	4	17	2	6	3	8	Location, GIS and weather data from sample collection points (domestic and imports)
2	1	3	1	1	3	8	Sampling protocol, analytical procedures and performance indicators
3	6	25	6	26			Identify and set maximum allowable levels
4	6	24			4	11	Data of packaging/storage/transport conditions
5			3	15	2	9	Health indicators <i>vis a vis</i> malnutrition
6			3	8	1	4	Food consumption data for various crops
7			3	5	1	5	Number of farmers producing at risk crops
8			1	1	2	3	Test data for imports collected at borders/customs and in the marketplace
9	●	●			2	4	Data on export demands/problems for/of dried fruits and nuts
10					5	21	Current level of contamination
11			5	18			Standard data collection and management system
12			4	11			Random sampling/retesting for validation
13	4	7					Inspection and expiration dates for products
14			3	6			Use data only from an accredited lab
15			2	5			Presence of mycotoxins at multiple points along the value chain
16			2	5			Presence of mycotoxins in different geographic areas (risk maps)
17	2	4					How crop is watered (rain-fed, river/lake, drip irrigation)
18	2	3					Healthy seed
19			2	3			Public Trade Law Enforcement Actions and track reports of corruption
20					1	1	Good Agricultural Practices – Pre-Harvest
21					1	1	Good Agricultural Practices – Post-Harvest
22					1	1	Farmer assistance to mitigate problem
23	●	●					Use of UV light to detect contamination (invalid technology)
24	●	●					Mycotoxin levels pre-harvest with supporting samples
25	●	●					Mycotoxin levels in stored products
26	●	●					Moisture content measurements
27	●	●					Data trends

Question T-3

Identify ways to increase the credibility of the results obtained from mycotoxin surveillance surveys in Afghanistan.

	Mountain		Ocean		Valley		Response
	^x #	^y S	#	S	#	S	
1	4	13	3	12	6	18	Good sampling plans
2	4	11	3	8	4	14	Capacity building of lab staff (analysis through reporting)
3	3	8			6	24	Accredited laboratory for chemical & biological assays
4			3	9	4	11	Use methods approved by International organizations such as USDA and FAO
5	2	8	1	4			Validated chemical/biological methods
6			1	4	2	6	Standard, high-quality equipment for conducting tests
7			5	25			Establish food safety standards for country
8	3	11					International cooperative projects
9					3	8	More public/engagement/awareness
10			3	8			Enforcement of mycotoxin limits
11			3	6			External and unannounced audits
12	3	4					Dissemination of results/publications in international outlets
13			2	8			Strong/sound government policies and commitment
14			2	7			Increased farmer awareness
15			2	5			Overall reduction in mycotoxins in all agricultural work
16			2	3			Quality assurance program
17			2	2			Continued training for lab staff to keep them up to date
18	1	4					Results from an accredited laboratory
19					1	3	Increased storage space for samples
20			1	3			High standards and quality control for work
21			1	2			International reputation for labs
22			•	•			Data transparently available to the public
23			•	•			Establish farmers' schools
24			•	•			Coordination amongst the stakeholders
25			•	•			Good Agricultural Practices – Pre-Harvest
26			•	•			Good Agricultural Practices – Post-Harvest
27					•	•	Sampling done by traders

Question 6-1

Identify methods and goals for inter-ministry collaboration on problems associated with mycotoxins in Afghanistan.

	City		Valley		Response
	^x #	^y S	#	S	
1	6	28	5	21	Define roles and responsibilities for each ministry
2	2	6	6	29	Interministerial/private sector task force with regular meetings and information sharing
3	2	2	6	28	Create national food safety standards and regulations
4	4	12	4	14	Joint unit/laboratory to monitor mycotoxins and associated problems
5	3	11	5	13	Align/synonymize ministerial policies
6	2	4	3	9	All parties commit resources
7	2	6	2	4	Define baselines
8	1	3	3	6	Increase public awareness
9	•	•	3	5	Border control of imported/exported goods with common rules across ministries
10	•	•	2	4	Develop institutional capacity of the ministries
11	2	3	•	•	Effective monitoring systems
12	1	5	•	•	Develop mitigation measures to reduce mycotoxin exposure
13			7	18	Increased surveillance throughout the country
14	2	7			Work plan agreed upon by all ministries
15	2	5			Develop coordination and collaboration mechanism for all stakeholders
16			2	5	Third party technical assistance
17	1	4			MAIL – Raw and unprocessed products
18	1	4			Provide information to private sector
19			1	4	Develop academic capacity
20			1	4	Establish pre/post-harvest management programs
21	1	2			Develop linkages between ministries
22	1	1			Establish and strengthen national CODEX committee for food safety management
23	1	1			Sampling, analyzing and reporting by MAIL
24	1	1			Manage standard irrigation methods
25			1	1	On line data sharing platform
26	•	•			Ministry of Commerce responsible for export/import licenses
27			•	•	Identify unified targets applicable to all three ministries
28			•	•	Effective communication
29			•	•	Create demand for clean food

Question 6-2

Identify regulations needed to limit mycotoxin exposure in Afghanistan.

	Lake		Mountain		Ocean		Response
	^x #	^y S	#	S	#	S	
1	4	18	6	28	6	29	Establish maximum residual levels in food and feed
2	2	6	4	16	3	8	Good Agricultural Practices – Pre-Harvest
3	1	3	●	●	3	5	Effective enforcement of established limits
4	2	7	4	7			Coordination of relevant ministries (Health, Agriculture, Trade & Finance)
5			2	7	2	8	National Research program on mycotoxins (academic)
6	●	●			4	14	Mycotoxin screening required of all food industries
7	2	7	2	3			Defined SOPs and inspection plans for critical control points
8			1	4	3	8	Good agricultural practices – Pre-Harvest
9					5	15	Establish a Food Safety Regulatory Authority
10	5	14					Assess capacity to enforce regulations
11			5	9			Government budget line to support enforcement
12					4	11	Implement food safety laws
13	4	9					Staff and private labs available to implement regulations
14	4	6					Surveillance of community markets
15			3	11			Standards for imports
16			3	11			Standards for exports
17					3	8	Political commitment to solve problem at a high level
18	3	6					Licensed private certification labs
19	3	6					Increased public awareness
20					2	9	Identify mycotoxins to be controlled
21					2	7	MOU for regional collaboration on mycotoxins
22	2	6					Inspection process for labs
23					2	5	Identify Maximum Tolerable Daily Intake based on Afghan diet
24					2	3	Increase/develop human and institutional capacity
25					2	3	Traceability and origin of foods and raw materials
26					2	2	Enforce Border Control on food migration/transport
27	1	4					Enable inspectors to work in a secure environment
28					1	3	Adopt international guidelines (CODEX)
29					1	3	Establish quarantine screen for imported foods
30			1	2			Trained food safety inspectors
31					1	2	Strengthen communication systems
32	1	1					Training for farmers and traders
33	●	●					Annual review of progress
34	●	●					Authorized testing program
35	●	●					Web site on regulations and how to meet them

Question 6-3

Identify cultural barriers to be overcome to reduce exposure to mycotoxins in Afghanistan.

	Mountain		Valley		Response
	^x #	^y S	#	S	
1	3	14	7	26	Traditional food processing/storage management/methods
2	4	9	2	8	Heavy reliance on wheat as a staple food
3			7	29	Food types/diversity
4	5	14			Limited education in farm households
5	5	14			Reliance on women to do much of this work
6	4	11			No knowledge of good agricultural practices, pre-harvest or post-harvest
7	4	11			Lack of marketing organizations
8			4	9	Local food preference over imported food
9			4	7	Trust and understanding of national authorities
10	3	11			Bad drying practices
11	3	9			Small farm size
12			3	9	Acknowledging the risk equals the word “dirty”
13			3	4	Food labeling
14	2	7			Limited cooperation among farmers and between associations
15	2	5			Mycotoxin risk will be new and unknown
16			2	5	Food taboos for children
17			2	2	Traditional weaning foods
18			1	4	Cooking styles/tastes
19	•	•			Need to incorporate other things into diet and not serve as primarily hospitality dishes

Question 6-4

Identify benefits resulting from lesser exposure to mycotoxins in Afghanistan.

	City		Lake		Valley		Response
	^x #	^y S	#	S	#	S	
1	6	30	7	35	4	17	Improved health
2	4	13	3	9	9	25	Increased economic growth/more jobs
3	3	6	4	8	4	6	Safe food more widely available
4	3	6	●	●	●	●	Post-harvest loss reduction
5			7	16	3	9	Better domestic/international markets
6	●	●	5	18			Reduced cost of medical treatment abroad
7			2	8	●	●	Improved product quality
8			2	5	●	●	Improved international reputation for exports
9			2	4	●	●	Increased productivity (not out sick)
10			2	2	●	●	Higher income for farmers/traders
11			1	4	●	●	Reduced childhood stunting
12			1	2	●	●	Reduced morbidity/mortality
13	7	23					Enables export promotion
14					5	23	International acceptance of certification of exports
15	4	12					Increase health of domesticated animals
16	4	6					Increased quantity of food available
17					3	9	Improved collaboration/communication between ministries
18					3	5	Increased scientific knowledge
19					2	8	Increased international investment
20			2	4			Fewer rejections of exported goods
21	1	3					Support for domestic markets
22			1	3			Provide confidence that the government can improve health
23	1	2					Increased confidence among farmers and exporters
24			●	●			More products that can be sold internationally
25			●	●			More markets for exports
26			●	●			Better equipped laboratories
27			●	●			Better trained laboratory staff
28					●	●	Reduced malnutrition
29					●	●	Improved public/private sector interactions
30					●	●	Increased foreign exchange
31					●	●	More sustainable growth
32					●	●	More sustainable growth

Question 8-1A

Who needs information on mycotoxins in Afghanistan?

	Lake		Ocean		Valley		Response
	^x #	^y S	#	S	#	S	
1	5	17	6	14	3	7	Farmers
2	1	1	8	28	3	10	Consumers/general public
3	4	14	5	19	1	5	Traders
4	1	3	3	8	3	13	Regulatory institutions/officials, <i>e.g.</i> , customs
5	4	15	●	●	1	5	Extension workers
6	5	25	8	36			Policy makers
7	4	9			2	7	Food processors
8			3	9	3	12	Ministries other than MAIL
9	5	8	●	●			Community decision makers
10	1	2			3	9	Health care providers
11	●	●			3	12	MAIL
12			5	12			Private sector
13	3	11					Lab staff
14	2	8					Staff at veterinary clinics
15	2	4					Religious leaders
16					2	4	Teachers/Educational institutions
17	2	3					NGOs
18			2	3			Legislators

Question 8-1B

How should information on mycotoxins in Afghanistan be delivered?

	Lake		Ocean		Valley		Response
	^x #	^y S	#	S	#	S	
1	1	1	8	40	4	19	Public media (radio, TV, print, <i>etc.</i>)
2	5	25	6	15	1	4	Official (govt.) publications and position papers
3	4	13	4	16	3	11	Social media
4	6	30	7	18			Workshops
5	7	18	3	9			Extension agents
6			3	7	1	5	Short term training
7	•	•	3	10			Official (govt.) web sites
8			1	3	•	•	Online sources
9	•	•	1	1			Word of mouth
10	6	15					Professional networks, trade publications and trade associations
11	6	11					MAIL
12			3	8			Community leaders
13					3	7	Mobile phone applications
14	2	7					Fliers
15			2	7			Information sharing events
16					2	7	Package labeling for consumers
17			2	3			Integrated Communications Technology (ICT)
18			1	2			Farmer cooperatives
19					1	2	Laboratory results
20			1	1			Agriculture fairs
21			•	•			Events where products are sold
22			•	•			Farmer to farmer
23					•	•	Comic books & posters
24					•	•	Mobile (traveling) theater

Question 8-2A

When should screening for mycotoxins occur in Afghanistan?

	City		Mountain		Response
	<i>x</i> #	<i>y</i> S	#	<i>S</i>	
1	5	18	4	15	Harvest
2	3	7	6	19	Drying/Processing
3	2	7	5	21	Prior to export
4	6	18	1	5	Warehouse/Storage
5	2	5	5	13	Storage/market place
6	4	4	●	●	Packaging
7	7	32			Pre-harvest in the field
8			5	21	Prior to import
9	5	18			Heading/grain filling
10			4	7	When the weather/conditions are favorable
11	3	7			Transportation from field to processor
12	3	5			Post-processing
13	●	●			Pre-processing

Question 8-2B

Where should screening for mycotoxins occur in Afghanistan?

	City		Mountain		Response
	<i>x</i> #	<i>y</i> S	#	<i>S</i>	
1	8	34	6	18	Preharvest – in the field
2	5	17	5	21	Customs/at the border
3	5	12	5	14	Market place
4	4	11	5	12	Laboratories
5	3	9	1	5	Post-harvest storage
6	6	18			Seed storage
7	4	10			Suspected locations
8			3	13	Processing/production site
9	3	6			Before/during transport
10			2	7	Humans/animals with biomarkers
11			2	4	Drying
12			2	4	For the crops at greatest risk
13	2	3			At home

Question 11-1A

Identify priorities for the next year for research on mycotoxins and potential applications of solutions in Afghanistan.

	City		Lake		Mountain		Ocean		Valley		Response
	^x #	^y S	#	S	#	S	#	S	#	S	
1	4	17	5	23			2	14	3	10	Establish inter-ministerial/private sector task force
2	2	14			1	4	5	11	3	9	Disseminate information on mycotoxins to the general population
3	1	4			3	20	8	40			National survey of mycotoxins
4	4	14			4	14	2	8			Government commitment/budget
5	2	14					7	28	1	3	Education for Afghan private sector on import restrictions elsewhere
6	2	5	5	22					2	8	Lab training for research and extension MAIL staff
7							7	28	1	4	User friendly manual for implementing new food safety law
8					4	25	3	18			Finalize food safety laws
9	4	14					3	10			Identify and begin working with donors
10	●	●					7	38			Establish mycotoxin regulations
11	2	14	4	18							Form consumer protection group
12	2	7							3	14	Identify MAIL/Commerce/Public Health/Private sector roles and responsibilities
13	2	5							3	14	Establish internal QA/QC labs for use by private sector
14	2	6			2	12					Develop strategies to minimize mycotoxin risks
15	1	4					3	11			Establish baseline for mycotoxin contamination
16	●	●					3	10			Develop and implement Good Agricultural Practices (post-harvest)
17	●	●					3	5			Develop and implement Good Agricultural Practices (pre-harvest)
18							7	31			Enhance laboratory testing quality control
19					6	21					Training courses for farmers
20			5	23							Form commodity association(s)
21			5	21							Develop HACCP baselines
22			5	17							Training for trade association members
23			5	12							Sensitize staff at all three ministries to the problem
24							4	22			Identify stakeholders

	City		Lake		Mountain		Ocean		Valley		Response
	^x #	^y S	#	S	#	S	#	S	#	S	
25	4	21									Infrastructure development
26							4	10			Improve knowledge sharing amongst stakeholders
27			3	18							Adapt manuals from California Pistachio Association for local use
28	3	12									Policy development
29	2	8									Implementation plan and budget by ministry
30			2	7							Laboratory manual with SOPs
31									1	7	Private sector trade missions to Afghanistan
32					1	5					Technical assistance for farmers
33	1	1									Implement mycotoxin control program
34	•	•									Send samples abroad for testing
35	•	•									Develop regulatory mechanisms
36							•	•			Identify products to use to reduce mycotoxin contamination
37									•	•	Local training for certification

Question 11-1B

Identify priorities for the next 5-10 years for research on mycotoxins and potential applications of solutions in Afghanistan.

	City		Lake		Mountain		Ocean		Valley		Response	
	^x #	^y S	#	S	#	S	#	S	#	S		
1			6	30	•	•	7	20	3	10	Accreditation for labs (public and private sector)	
2	2	5	4	22			4	20	•	•	Human and Institutional Capacity Development (HICD)	
3	•	•			2	8	4	11			Develop and implement Good Agricultural Practices – pre-harvest & post-harvest Improve infrastructure (labs) Develop mycotoxin mitigation strategies Adopt standards for maximum contamination allowed Establish regional labs for surveillance Government funding Design and implement food safety (HAACP) interventions Establish food safety authority Local training for certification Sustainable system for oversight and enforcement Better testing/documentation for exports	
4	4	21					7	30				
5	3	7					8	42				
6					2	8	9	37				
7	1	5					9	44				
8	4	14	4	22								
9			5	23					3	13		
10					5	12	3	8				
11	5	18							•	•		
12	•	•	4	22								
13	•	•							3	16		
14							6	21				Establish national policies Modules/courses in degree programs SOPs for all toxins along value chains National surveillance and data collection program Training for research & extension staff Identify alternative uses/markets for contaminated products Donor funding Strengthen border control/quarantine Establish independent legal enforcement body MAIL/Commerce/Private sector working group Public awareness campaign(s) Organize/strengthen farmer's cooperatives Communication strategy Promote healthy diets and food choices MAIL/Ministry of Public Health monitor-
15			6	20								
16			6	16								
17			5	17								
18			4	22								
19							4	15				
20	4	14										
21							4	14				
22							3	16				
23									3	10		
24									3	9		
25					3	4						
26	2	14										
27									1	7		
28									1	3		

	City		Lake		Mountain		Ocean		Valley		Response
	^x #	^y S	#	S	#	S	#	S	#	S	
29									1	3	ing strategy Implementable control strategy from inter-ministerial/private sector collaborations

Notes:

^x# – Number of persons ranking this response as one of the five (seven, for questions 11-1A and 11-1B) most important.

^yS – Weighted priority score, with each voting member ranking their top five topics. Five points assigned to the most important response and one point to the least significant of the important responses. For questions 11-1A and 11-1B, seven points and seven topics were selected instead of five.

^z● – This response provided by one or more members of the group, but not identified as amongst the five or seven most important responses by any member of the group.

“ ” – This response not provided by any member of the group.

**Appendix XIII –
Leslie PowerPoint presentation at USAID Kabul – 17 March 2016**

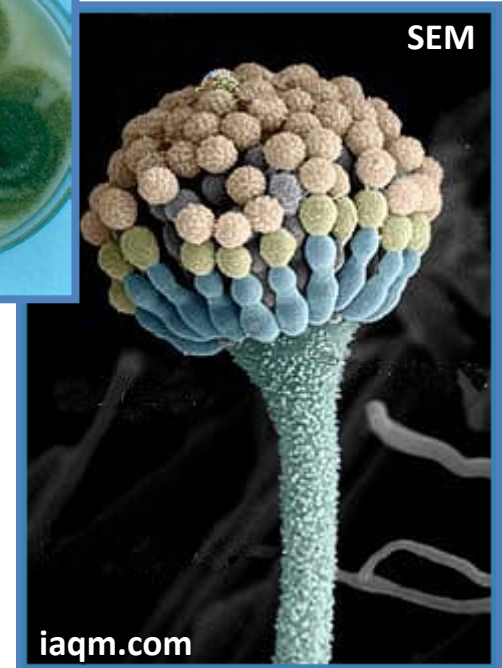
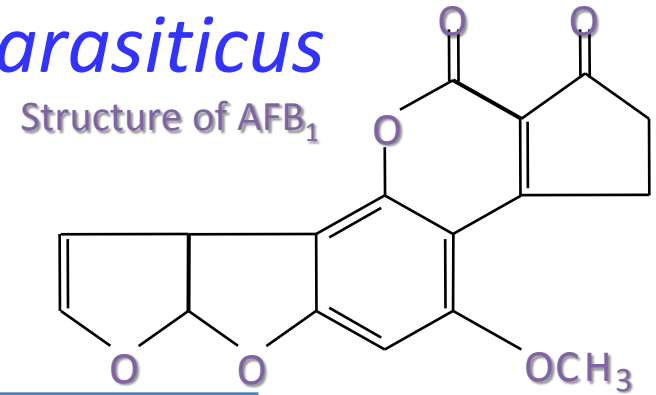
Where Do We Go Now?

John Leslie

John Floros

Aflatoxins

Aspergillus flavus / *A. parasiticus*



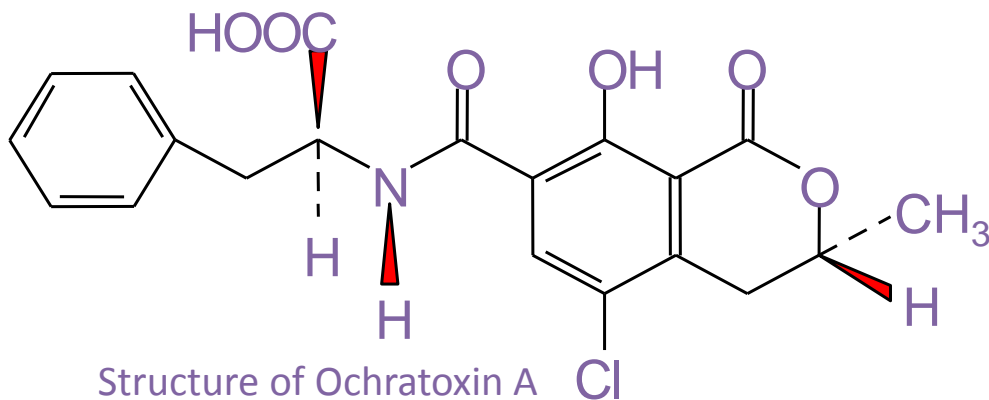
Liver failure
Liver cancer
Growth stunting
Immune deficiency or
suppression
Grains – especially maize
Peanuts
Nuts

Ochratoxins

Aspergillus ochraceus



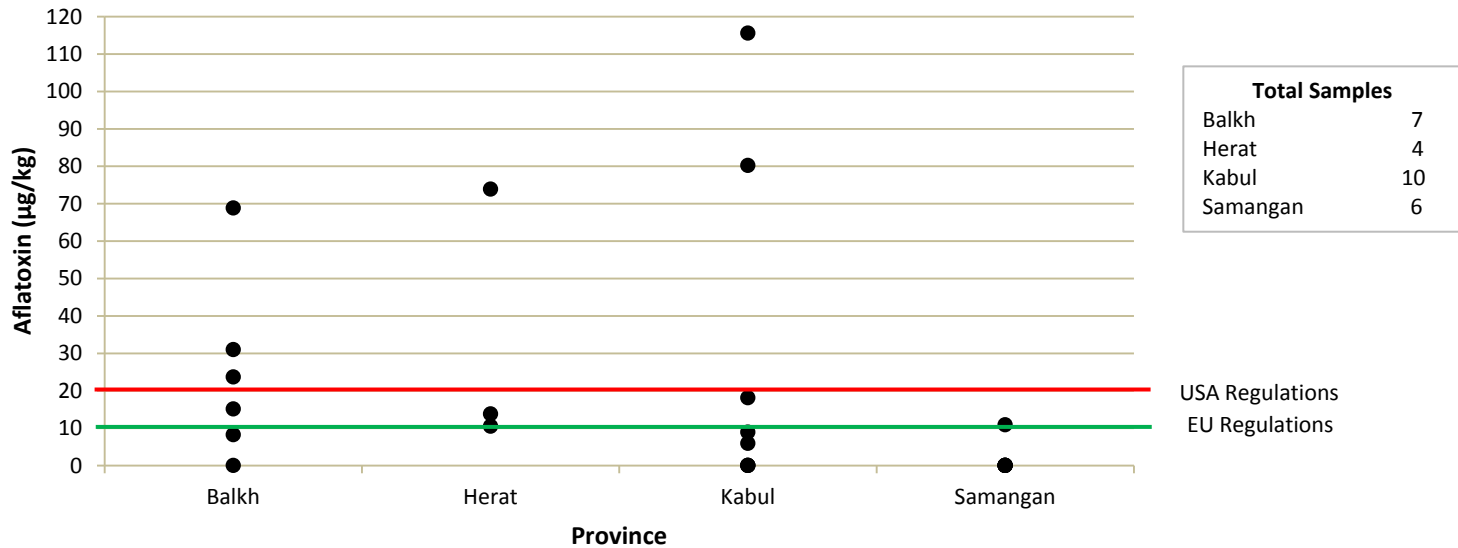
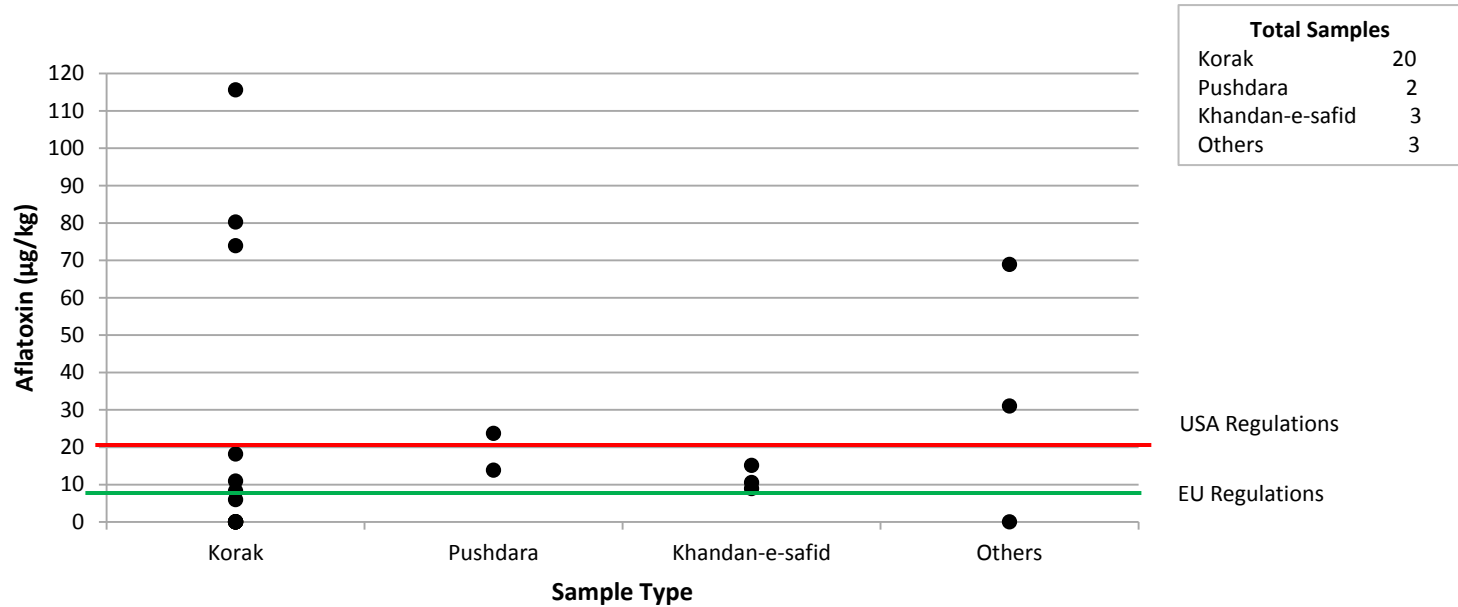
Kidney failure
Cacao
Nuts
Grapes
Coffee
Wheat



Nuts – Results

- Aflatoxin
 - Almonds – 15/81 at export limiting level
 - Pistachios – 19/40 at export limiting level
 - Walnuts – 8/25 at export limiting level
- Ochratoxin
 - Almonds – no contamination detected
 - Pistachios – 2/40 at export limiting levels
 - Walnuts – no contamination detected

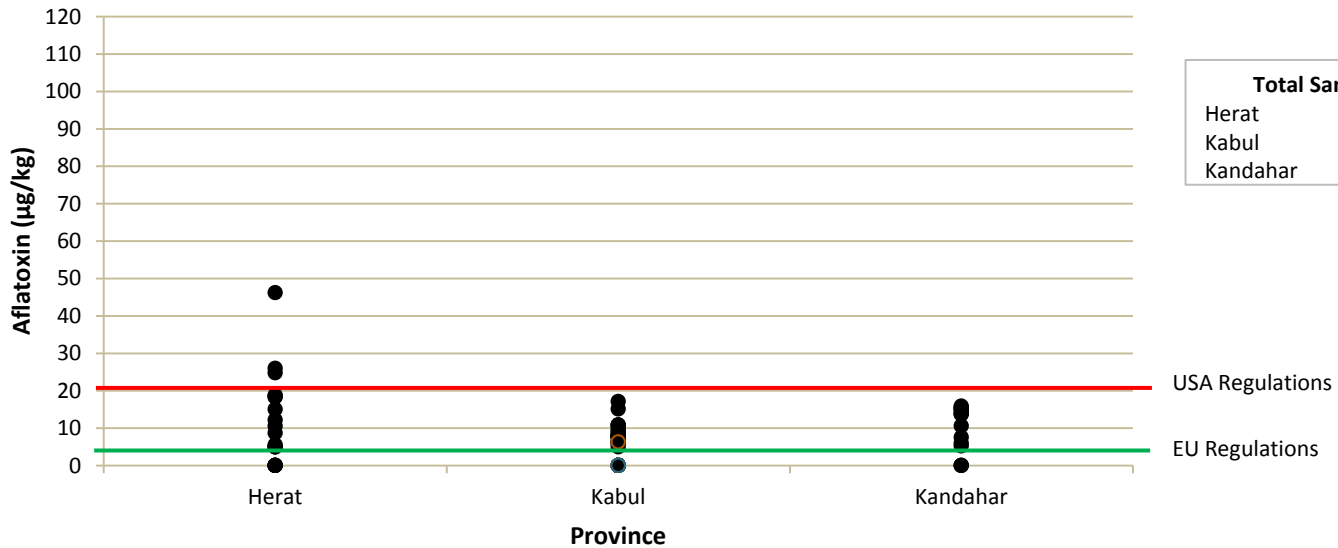
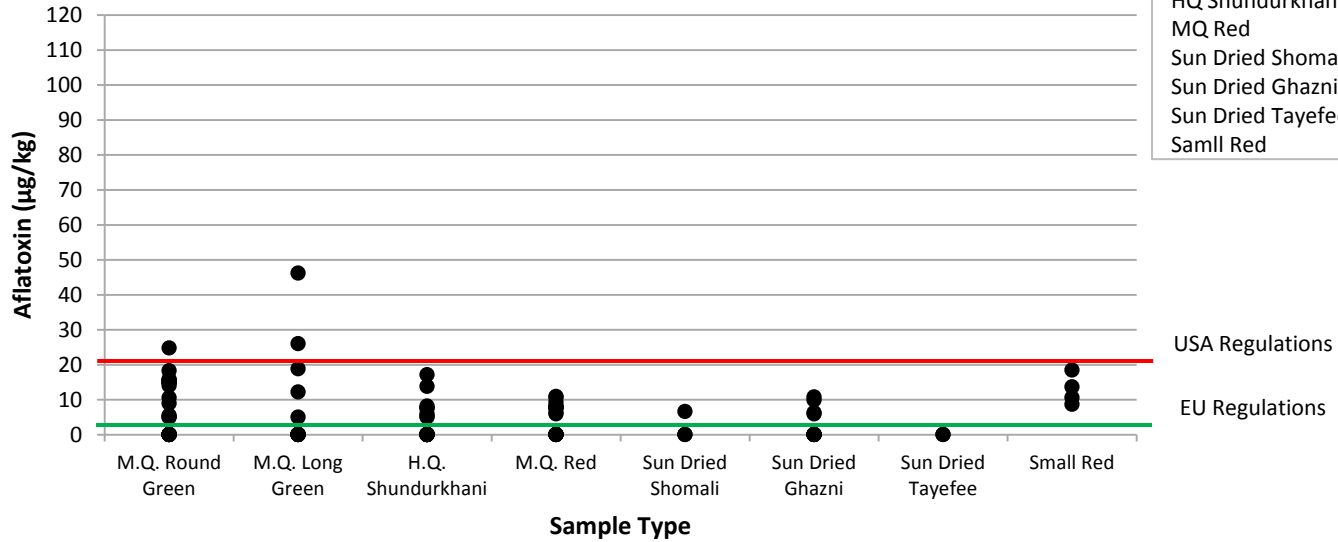
Pistachios – Aflatoxins



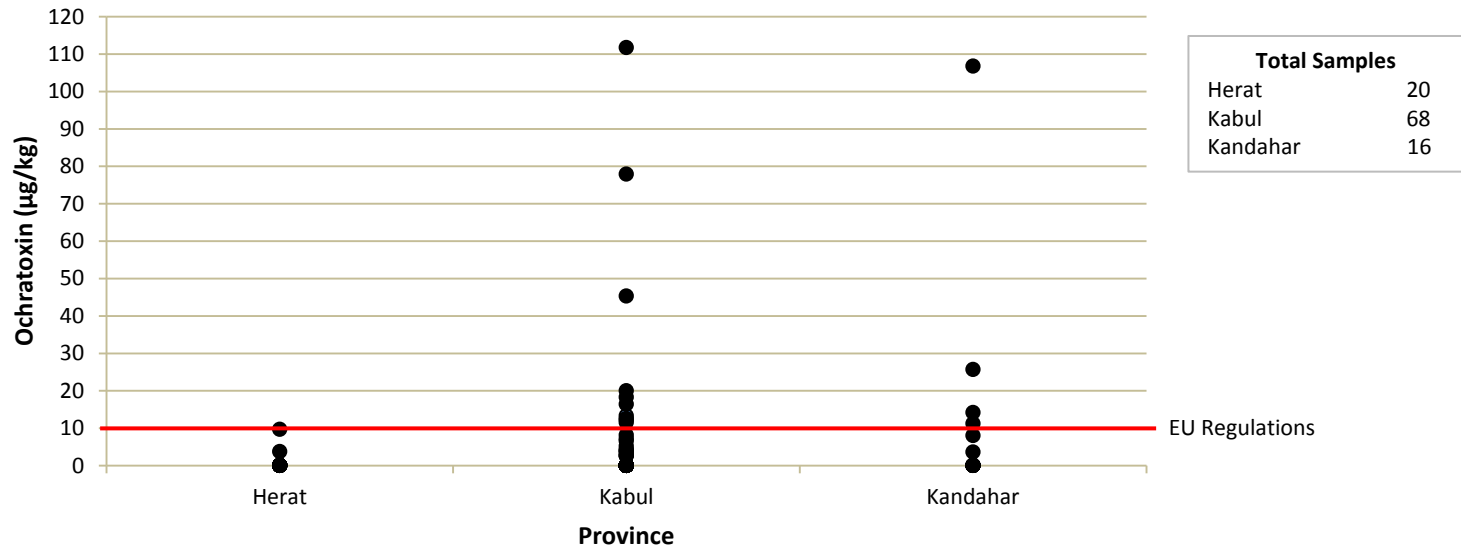
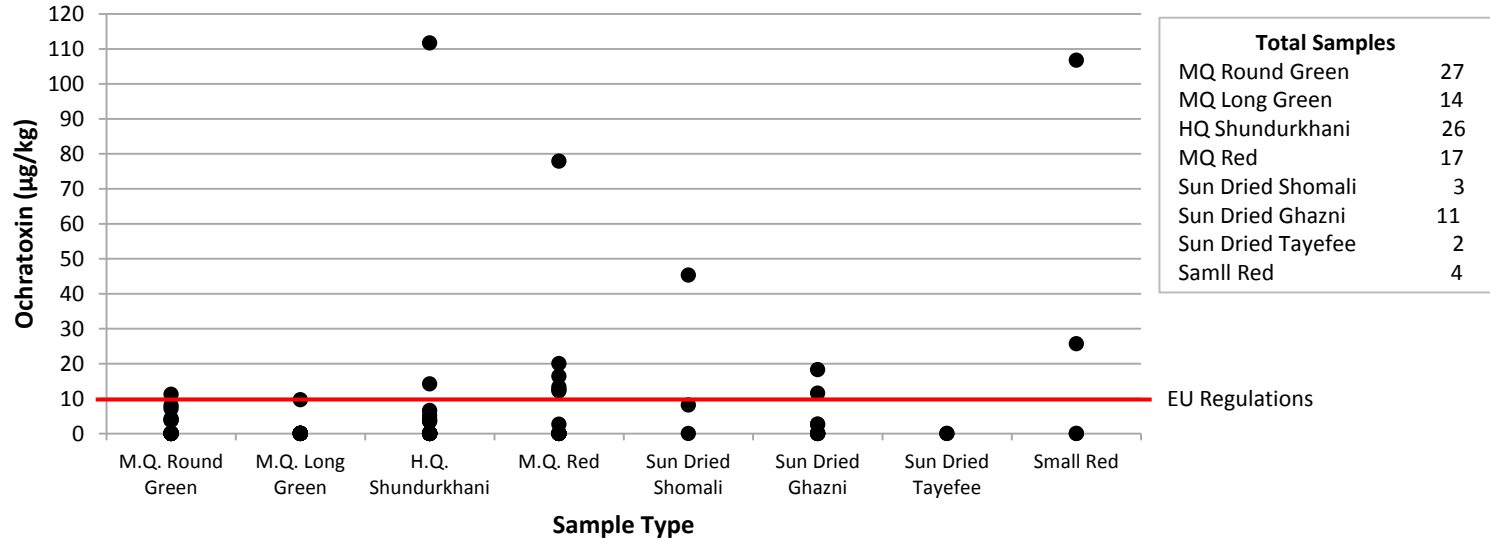
Raisins – Results

- Aflatoxins could limit exports in 43/89 samples
- Ochratoxin could limit exports in 25/80 samples
- Raisin type and drying method can be important
- Afghanistan and Austria results are discordant
- Choice of country to export to may depend on level of contamination

Raisins – Aflatoxins



Raisins – Ochratoxin

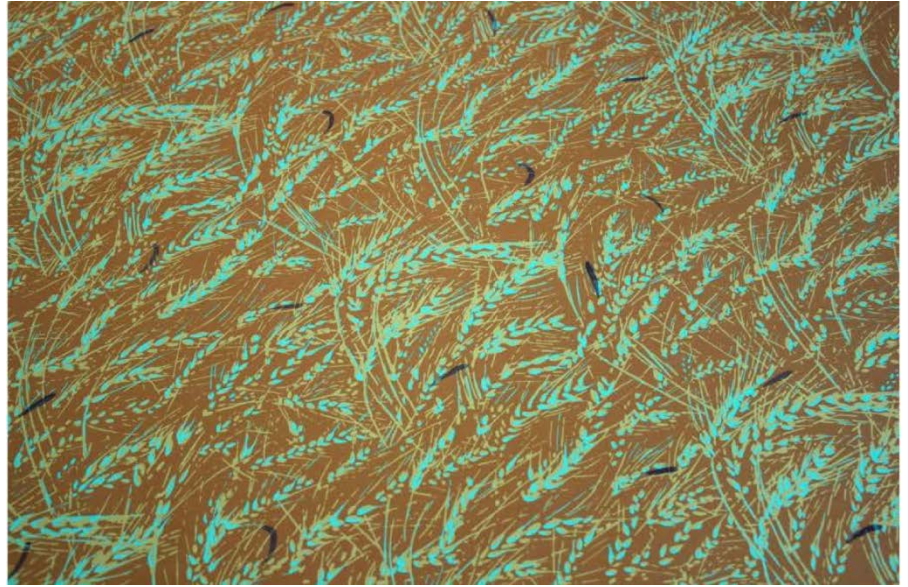


Trichothecenes

- Two classes – A & B, strains make only one type
- Both inhibit protein synthesis
- Most common in grains
- Type A – very toxic – T-2, HT-2 & DAS
 - US Select agent list
 - Purportedly used for biological warfare
- Type B – not as toxic – DON, NIV
 - More widespread, especially on wheat
- Can be taken up through skin or intestinal mucosa
- Cause vomiting, diarrhea, & immune suppression

Ergot Alkaloids

- Small Grains – Wheat, rye, barley & oats
- An unexpected finding by Austrian group
- Not highly regulated (animal feed only)
- In small doses – hallucinations (LSD)
- In other cases – neuropathy and gangrene
- Gnostics and ancient Greeks may have used them to help people have visions
- Controlled by sorting ergot bodies from the grain before processing



Some Ergot Epidemics

-600 – Assyria

857 – Germany

945 – France

1093 – France

1692 – USA

1926 – Russia

1929 – Ireland

1953 – France

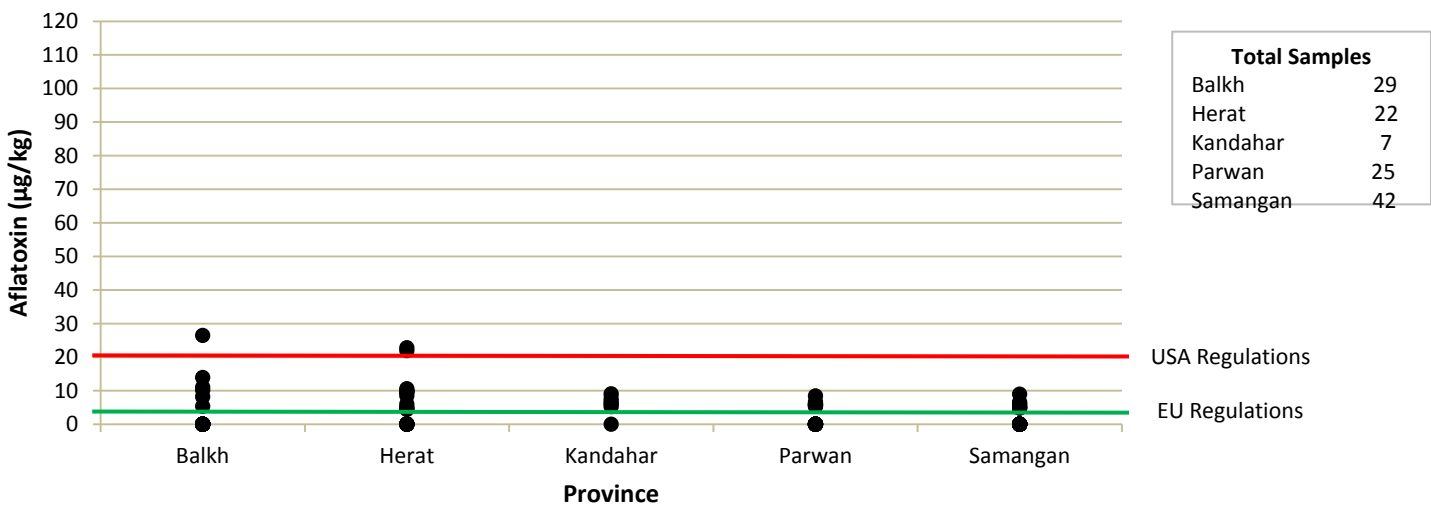
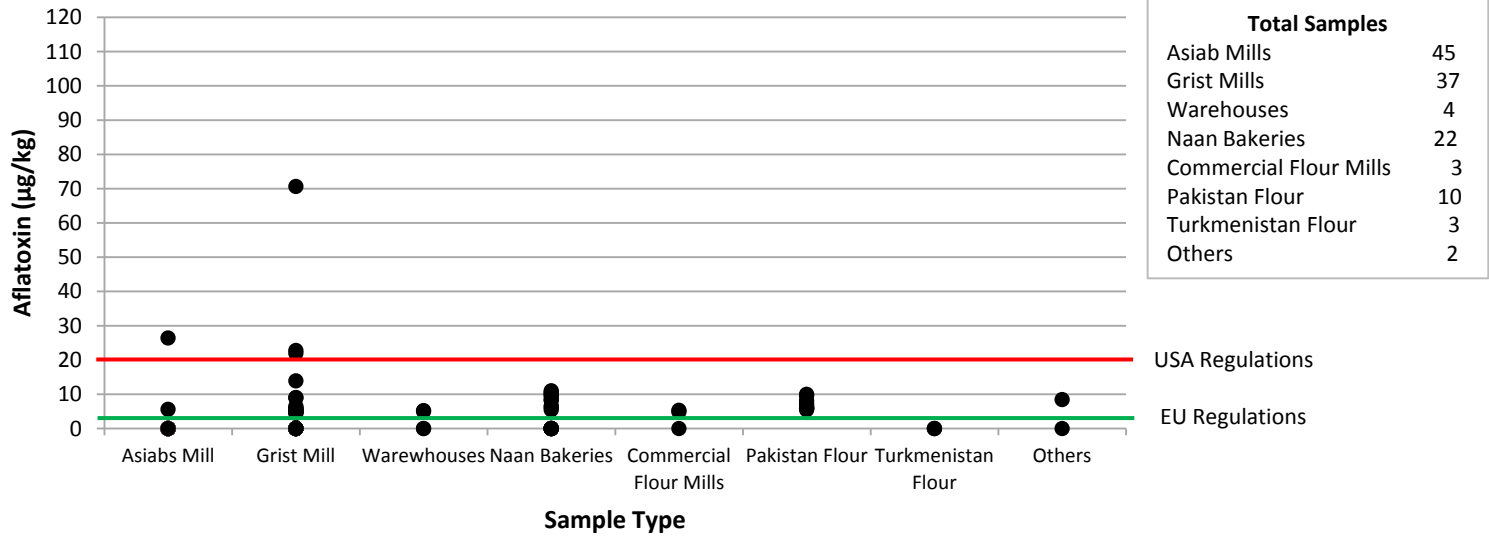
1958 – India

1973 – Ethiopia

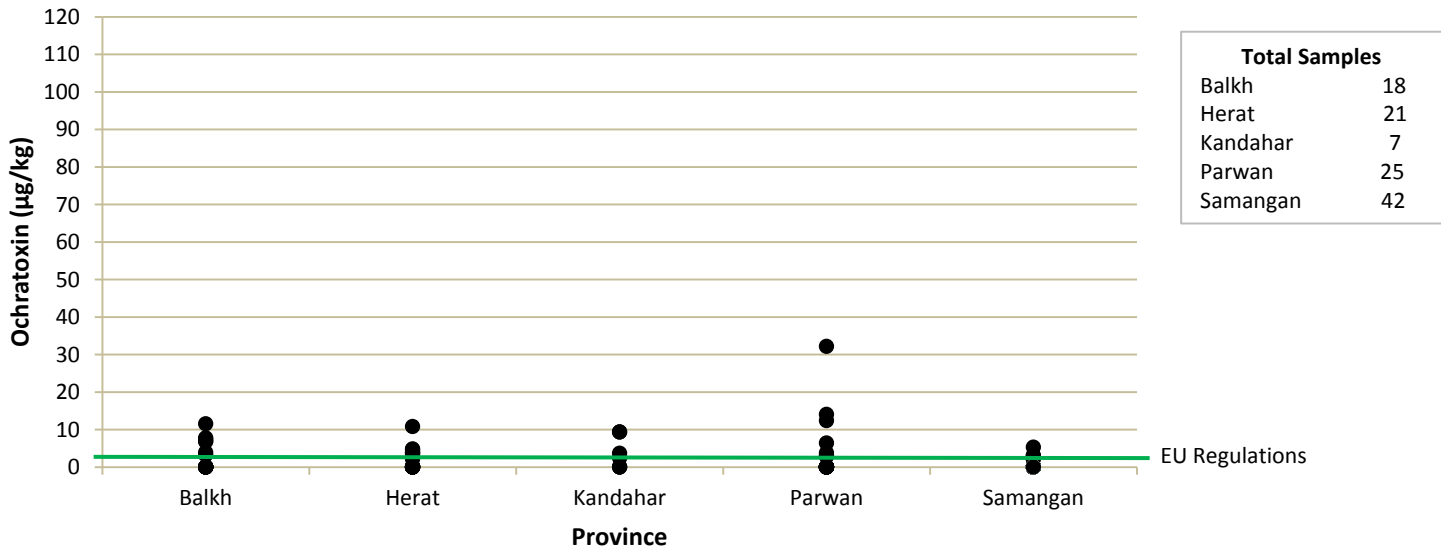
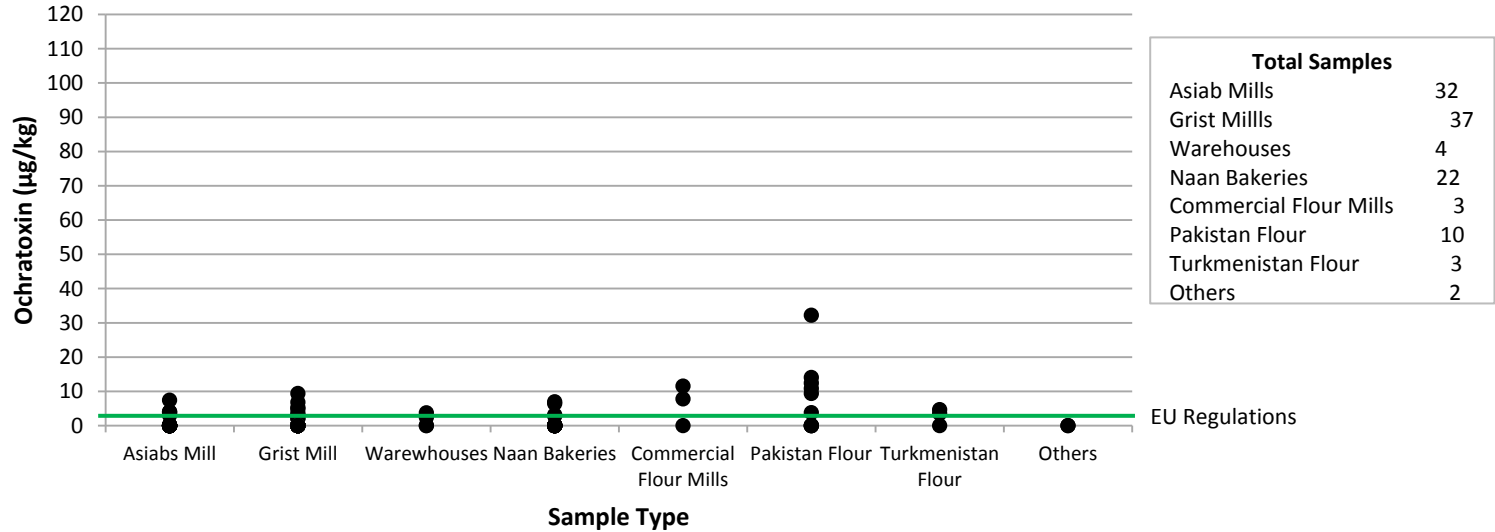
Wheat – Results

- International standards may be too high for Afghanistan safety because of the large amount of wheat consumed daily (500 g/person/day)
- Aflatoxins – detectable in 23/151 samples
 - Not a field contaminant of wheat
 - Contamination likely occurs in storage
- DON – 3/185 above international guidelines
 - Not a major problem, but exists
 - Weather and storage dependent
- Ochratoxin – detectable in 36/181
 - Common problem in northern Europe
 - Needs attention
 - May carry over to meat
- T-2 and HT-2 – Not reliably detected
- Ergot – detectable 51/151
 - High incidence
 - Easily remedied by cleaning grain

Wheat - Aflatoxin



Wheat – Ochratoxin



Austrian Screen – Wheat

<i>Fusarium</i>	<i>Alternaria</i>	<i>Aspergillus</i>	<i>Penicillium</i>	<i>Claviceps</i>
Beauvericin	Alternariol	Aflatoxin	Agroclavine	Ergocristine
Enniatin A	Alternariol methyl ether	Averantin	Chanoclavine	Ergocristinine
Enniatin A ₁	Altersetin	Averufin	Citrinin	Ergometrine
Enniatin B	Altersolanol	Cycloaspeptide A	Chrysogine	Ergometrinine
Enniatin B ₁	Altertoxin-I	Kojic acid	Elymoclavine	Ergosin
Epiequisetin	Macrosporin	Methoxysterigm atocystin	Mycophenolic acid	Ergosinin
Equisetin	Tentoxin	3-Nitropropionic acid	Questiomycin A	Ergotamine
HT-2 toxin	Tenuazonic acid	Norsolorinic acid	Quinolactacin A	Ergotaminine
T-2 toxin		Ochratoxin	Secalonic acid D	
Zearalenone		Sterigmatocystin		

Efforts for This Week and This Month

- Synthesize nominal group results and return to Mac.
- Develop teams in Afghanistan to make decisions and help design implementation strategies.
- Identify appropriate international partners
- Help the effort grow to a food safety effort that is broader than just mycotoxins

What Are Some Problems?

- Confidence in Data Collection and Results
 - Training & Education
 - Facilities
 - Communication
 - Politics
-
- Action Items and Next Steps

A Few of Our Ideas

- Separate research (information gathering) from regulation
- Involve university staff and students
- Collect baseline data
- Routine back up to confirm controversial results
- Become part of the international conversation on mycotoxins
- Take advantage of your uniqueness
- Steal good ideas shamelessly (with credit)

A Few Examples of Actions

- Short Term
 - Train people in Food Safety Principles
 - Clean up the Ergot
 - Identify Alternative Markets for products
- Long Term
 - Develop Communication Strategies for ...
 - Train MS & PhD level scientists
 - Develop Import Standards and Regulations

Round 1

- Short term
 - National Survey of mycotoxins
 - Food Safety Laws
 - Form Commodity Associations
 - Infrastructure development
 - Identify ministry roles in mycotoxins
- Long term
 - Regional mycotoxin labs
 - Organize a Food Safety Authority
 - Government certification of private labs
 - Technical help for the private sector
 - Promotion of healthy food

Round 2

- Short Term
 - Create private sector labs accredited by the government
 - Training courses for farmers
 - Create inter-ministerial working groups
 - Find Money - Donors
 - Coordinate Ministries and ACCI
 - Establish mycotoxin regulations
- Long Term
 - Dietary Guidelines
 - Good Pre- and Post-Harvest Practices
 - Find money – Government
 - HACCP studies of each commodity and forest products
 - Develop mitigation strategies for mycotoxins

Appendix XIV –

Excel spreadsheet comparing results from different tests/institutions

XIV.1 Sheet 1 – Raisins

XIV.2 Sheet 2 – Almonds

XIV.3 Sheet 3 – Pistachios

XIV.4 Sheet 4 – Walnuts

XIV.5 Sheet 5 – Aflatoxin in Wheat

XIV.6 Sheet 6 – Deoxynivalenol in Wheat

XIV.7 Sheet 7 – T-2 Toxin in Wheat

Random Number	Sample ID	Aflatoxin		Ochratoxin	
		Afghanistan Romer	Austria	Afghanistan Romer	Austria
259	R07-H-S2-8-9-15-010-002	< LOD	< LOD	< LOD	< LOD
266	R07-H-S1-8-9-15-007-007	< LOD	< LOD	< LOD	< LOD
269	R01-H-S4-8-12-2015-052-005	18	< LOD	4	< LOD
296	R04-KBL-S1-10-3-15-325-008	9	< LOD	13	< LOD
298	R02-H-S2-8-10-15-056-002	46	< LOD	< LOD	< LOD
300	R03-KBL-S1-10-14-15-369-017	< LOD	< LOD	< LOD	< LOD
301	R01-H-S1-8-12-2015-053-003	15	< LOD	Trace	< LOD
302	R08-H-S2-8-9-15-011-001	9	< LOD	< LOD	10
303	R08-H-S3-8-9-15-0020-002	18	< LOD	< LOD	< LOD
304	R02-H-S3-8-11-15-0067-004	Trace	< LOD	< LOD	< LOD
306	R02-H-S4-8-10-15-059-003	< LOD	< LOD	< LOD	< LOD
307	R02-H-S4-8-11-2015-072-001	12	< LOD	10	< LOD
309	R01-H-S2-8-11-15-012-007	5	< LOD	< LOD	< LOD
310	R02-H-S2-8-12-15-015-005	5	< LOD	< LOD	< LOD
311	R01-H-S3-8-9-15-013-006	6	< LOD	< LOD	< LOD
312	R01-H-S3-8-12-2015-014-002	25	< LOD	< LOD	< LOD
314	R03-KBL-S1-4-10-15-344-022	< LOD	< LOD	5	< LOD
322	R03-KBL-S1-4-10-15-382-021	5	< LOD	< LOD	< LOD
323	R02-H-S2-8-11-15-091-008	19	< LOD	< LOD	< LOD
325	R03-KBL-S1-10-3-15-333-012	< LOD	< LOD	< LOD	< LOD
327	R04-KBL-S1-4-10-15-381-010	8	< LOD	13	< LOD
329	R03-KBL-S1-10-4-2015-360-019	< LOD	< LOD	< LOD	< LOD
330	R05-KBL-S1-4-10-2015-375-024	< LOD	< LOD	45	< LOD
333	R03-KBL-S1-10-3-15-321-010	< LOD	< LOD	< LOD	< LOD
334	R01-KN-S2-9-9-15-0224-009	15	< LOD	< LOD	< LOD
335	R01-KBL-S1-4-10-15-390-018	< LOD	< LOD	< LOD	< LOD
337	R01-KBL-S1-4-10-15-377-017	< LOD	< LOD	< LOD	< LOD
338	R02-KBL-S1-10-11-2015-454-013	< LOD	< LOD	< LOD	< LOD
340	R01-KN-S1-9-9-15-0225-0012	14	< LOD	< LOD	< LOD
342	R04-KBL-S1-10-4-15-342-006	8	< LOD	< LOD	14
344	R08-KN-S2-9-8-15-227-004	14	< LOD	26	< LOD
347	R04-KBL-S1-10-5-15-395-012	6	< LOD	16	< LOD
348	R03-KN-S1-9-8-15-2015-005	< LOD	< LOD	14	< LOD
350	R04-KBL-S1-4-10-15-386-011	8	< LOD	20	1
351	R03-KBL-S1-10-3-15-334-008	< LOD	< LOD	112	< LOD
352	R01-KBL-S1-4-10-15-362-023	< LOD	< LOD	< LOD	< LOD
355	R03-KBL-S1-03-10-15-322-009	8	< LOD	< LOD	< LOD
356	R04-KBL-S1-10-11-15-458-007	< LOD	< LOD	78	< LOD
361	R03-KBL-S1-10-3-15-361-015	< LOD	< LOD	< LOD	< LOD
369	R01-KN-S2-9-9-15-0223-0010	16	< LOD	8	< LOD
370	R05-KBL-S1-4-10-2015-389-025	7	< LOD	< LOD	< LOD
372	R03-KN-S1-9-8-15-216-002	6	< LOD	149	< LOD
374	R06-KBL-S1-10-10-15-428-022	6	< LOD	< LOD	< LOD
379	R03-KBL-S1-10-4-2015-384-020	< LOD	< LOD	< LOD	< LOD
381	R01-KBL-S1-4-10-15-351-021	< LOD	< LOD	< LOD	< LOD
382	R04-KBL-S1-10-4-15-391-009	8	< LOD	12	< LOD
383	R03-KBL-S1-10-3-15-324-007	17	< LOD	< LOD	< LOD
384	R01-KBL-S1-4-10-15-368-019	Trace	< LOD	4	1
385	R06-KBL-S1-10-5-15-399-003	11	< LOD	18	< LOD
386	R03-KBL-S1-10-3-15-329-014	< LOD	< LOD	< LOD	< LOD
390	R03-KBL-S1-4-10-15-353-021	< LOD	< LOD	4	< LOD
391	R06-KBL-S1-10-10-15-434-023	< LOD	< LOD	3	< LOD
392	R01-KBL-S1-4-10-15-355-020	< LOD	< LOD	4	< LOD
395	R03-KBL-S1-10-3-15-316-011	< LOD	< LOD	< LOD	< LOD
401	R06-KBL-S1-10-5-15-394-005	10	< LOD	< LOD	< LOD
402	R03-KN-S2-9-9-15-0212-001	14	< LOD	4	61
405	R04-KBL-S1-4-10-2015-356-002	< LOD	< LOD	3	< LOD
406	R06-KBL-S1-10-10-15-419-025	< LOD	< LOD	3	< LOD
407	R06-KBL-S1-10-10-15-422-026	< LOD	< LOD	< LOD	< LOD
408	R02-H-S4-8-11-15-0066-001	< LOD	< LOD	< LOD	< LOD
409	R01-H-S1-8-12-2015-0073-004	11	< LOD	< LOD	< LOD
410	R04-KBL-S1-10-4-15-350-004	8	< LOD	< LOD	< LOD
411	R01-KBL-S1-4-10-15-357-015	< LOD	< LOD	< LOD	< LOD
412	R01-H-S4-8-12-2015-009-001	5	< LOD	< LOD	< LOD
413	R01-KBL-S1-4-10-15-385-024	< LOD	< LOD	< LOD	< LOD
414	R01-KBL-S1-4-10-15-349-014	15	< LOD	< LOD	< LOD
419	R04-KBL-S1-10-11-15-444-006	< LOD	< LOD	< LOD	< LOD
420	R03-KN-S1-9-10-15-238-006	5	< LOD	< LOD	3
421	R06-KBL-S1-10-3-15-313-019	< LOD	< LOD	< LOD	< LOD
432	R06-KBL-S1-10-10-15-418-021	< LOD	< LOD	< LOD	< LOD
435	R04-KBL-S1-10-11-15-451-008	6	< LOD	Trace	< LOD
436	R06-KBL-S1-10-10-15-416-024	< LOD	< LOD	< LOD	< LOD
437	R04-KBL-S1-10-4-15-346-005	11	< LOD	< LOD	< LOD
438	R04-KBL-S1-10-11-15-453-004	< LOD	< LOD	< LOD	< LOD
439	R01-KBL-S1-3-10-15-332-026	< LOD	< LOD	< LOD	< LOD
441	R03-KBL-S1-10-3-15-315-016	< LOD	< LOD	3	< LOD
442	R02-KBL-S1-10-11-2015-446-009	< LOD	< LOD	< LOD	< LOD
443	R02-KBL-S1-10-11-2015-449-012	Trace	< LOD	< LOD	< LOD
444	R06-KBL-S1-10-10-15-437-020	< LOD	< LOD	< LOD	< LOD
445	R08-KN-S2-9-9-15-226-003	11	< LOD	107	59
446	R03-KBL-S1-10-5-15-398-022	8	< LOD	7	< LOD
447	R01-KN-S1-9-9-15-0222-0013	16	< LOD	< LOD	< LOD
448	R02-KBL-S1-10-11-2015-441-011	< LOD	< LOD	< LOD	< LOD
450	R04-KBL-S1-10-4-15-363-007	11	< LOD	< LOD	< LOD
451	R04-KBL-S1-10-11-15-455-005	< LOD	< LOD	< LOD	< LOD
453	R03-KN-S1-9-9-15-215-004	8	< LOD	< LOD	< LOD
454	R02-KBL-S1-10-10-2015-412-010	< LOD	< LOD	< LOD	< LOD
458	R01-KN-S2-9-9-15-214-011	15	< LOD	11	< LOD
459	R03-KN-S2-9-9-15-217-003	Trace	< LOD	< LOD	< LOD

Aflatoxin	Ochratoxin
< 4.0	< 2.0
> 4.0 - 10.0	> 2.0 - 5.0
> 10.0 - 20.0	> 5.0 - 10.0
> 20.0	> 10.0

Random Number	Sample ID	Aflatoxin
		Afghanistan Romer
600	P03-KBL-S1-10-4-15-392-003	9
604	P01-KBL-S1-10-11-15-456-005	116
606	P01-KBL-S1-10-11-15-445-003	18
607	P01-KBL-S1-10-10-15-432-004	< LOD
610	P01-S-S1-10-30-2015-058-015	< LOD
611	P04-B-S5-8-4-2015-076-020	< LOD
612	P01-S-S1-10-30-2015-067-016	< LOD
614	P01-S-S1-10-31-2015-079-017	11
618	P01-B-S2-8-3-15-018-009	8
622	P01-KBL-S1-10-10-15-439-0011	184
624	P01-S-S1-10-31-2015-089-021	< LOD
628	P04-B-S4-8-4-15-075-002	31
629	P01-S-S1-10-31-2015-088-018	< LOD
632	P02-B-S2-8-3-2015-008-002	24

Aflatoxin		
UNL Romer	UNL Neogen	Austria
< LOD	< LOD	< LOD
12	13	1189
9	6	5
< LOD	< LOD	1
< LOD	20	< LOD
< LOD	< LOD	< LOD
46	29	142
< LOD	< LOD	< LOD
< LOD	6	0.4
< LOD	< LOD	1
100	95	14
77	82	82
Trace	Trace	< LOD
27	24	14

Aflatoxin
< 4.0
> 4.0 - 10.0
> 10.0 - 20.0
> 20.0

Random Number	Sample ID	Aflatoxin	Aflatoxin		
		Afghanistan Romer	UNL Romer	UNL Neogen	Austria
551	WN01-S-S1-10-31-15-085-005	25	< LOD	< LOD	< LOD
554	WN01-S-S2-10-31-15-070-006	22	< LOD	< LOD	< LOD
556	WN05-KBL-S1-10-11-15-442-003	< LOD	< LOD	< LOD	< LOD
557	WN03-B-S4-8-4-15-077-001	Trace	< LOD	< LOD	< LOD
578	WN06-KBL-S1-10-10-15-430-005	< LOD	8	< LOD	< LOD
579	WN05-KBL-S1-10-10-15-407-09	< LOD	< LOD	< LOD	< LOD
580	WN06-KBL-S1-10-10-15-413-008	6	Trace	< LOD	< LOD
581	WN05-B-S1-8-4-15-073-007	< LOD	12	< LOD	< LOD
582	WN06-B-S4-8-14-15-041-003	42	< LOD	< LOD	< LOD
583	WN06-H-S5-8-4-15-022-001	40	< LOD	< LOD	< LOD
584	WN01-KBL-S1-10-11-15-459-002	< LOD	5	< LOD	< LOD
585	WN05-KBL-S1-10-3-15-326-001	37	< LOD	< LOD	< LOD
587	WN06-B-S3-8-3-15-046-004	41	Trace	< LOD	< LOD
589	WN05-KBL-S1-10-10-15-408-008	< LOD	Trace	< LOD	< LOD
590	WN06-KBL-S1-10-3-15-303-014	11	Trace	< LOD	< LOD
594	WN01-KBL-S1-10-10-15-438-003	< LOD	< LOD	< LOD	< LOD
595	WN05-KBL-S1-10-3-15-320-002	41	11	< LOD	< LOD
596	WN05-KBL-S1-10-4-15-373-005	< LOD	Trace	< LOD	< LOD

Aflatoxin

< 4.0
> 4.0 - 10.0
> 10.0 - 20.0
> 20.0

**Appendix XV –
Materials from Kabul Risk Communication meeting – 16 July 2016**

XV.1 Draft Scope of Work – 14 June 2016

XV.2 KSU Response to Draft Scope of Work – 6 July 2016

XV.3 Workshop agenda

XV.4 Workshop participants

XV.5 Questions for small group discussions

XV.6 Leslie – Project results PowerPoint

XV.7 Leslie – Delhi meeting summary PowerPoint

XV.8 Cosic – Communications PowerPoint

DRAFT Scope of Work (14 June 2016)

Background:

In December 2015, Afghanistan's Ministry of Agriculture, Irrigation and Livestock (MAIL), with support from the United States Agency for International Development (USAID), opened a laboratory to inspect Afghan agricultural products for mycotoxins, toxic byproducts of mold. USAID also partnered with MAIL to conduct a rapid assessment of mycotoxins in Afghan agricultural products between XXXX-XXXX. The rapid assessment found that there is a prevalence of mycotoxins in Afghan agricultural products. USAID hosted a conference in New Delhi on XXXX to determine next steps.

USAID is requesting support from Kansas State University to provide strategic communications guidance and capacity building support for MAIL to effectively and accurately communicate to Afghan citizens, government counterparts, and the international community concerning the prevalence of mycotoxins in Afghan agricultural products, the dangers they pose, and the strategy MAIL has developed to ensure that Afghan agricultural products are safe for export and consumption.

Deliverables:

Kansas State University, working in conjunction with the USAID Office of Agriculture (OAG) and the Development Outreach and Communications Office (DOC), will arrange for a strategic communications advisor to provide communications guidance and capacity building support for MAIL based upon the strategy that MAIL has developed to ensure that Afghan agricultural products are safe for export and consumption. This guidance and capacity building support can include but is not limited to:

- Guidance on the development of a strategic communications plan based upon the strategy that MAIL has developed to ensure that Afghan agricultural products are safe for export and consumption. This plan should include a methodology and proposed timeline of how best to inform relevant audiences and to keep these audiences informed as the implementation of MAIL's strategy progresses. This plan should also include performance benchmarks that can be monitored and evaluated;
- Guidance on monitoring of domestic and international traditional media and social media concerning mycotoxins and MAIL's ability to address the problem in English, Dari, and Pashto;
- The polling of target audiences over the span of the implementation of the communications plan to track changes in opinion and understanding of the issues;
- Guidance on the organization of press conferences, media roundtables, meetings and townhalls at the national, provincial, and community level to inform and educate the public, farmers, and agribusinesses on how to ensure the safety of Afghan agricultural products that will be delivered and/or simultaneously translated into English, Dari, and Pashto;
- Guidance on the advance drafting and translating of talking points, press releases, updates, and social media toolkits to be shared by MAIL and other Afghan government stakeholder in English, Dari, and Pashto;

- Guidance on the production of radio and television PSAs to be broadcast across the country in English, Dari, and Pashto;
- Guidance on the oversight of social media communications in English, Dari, and Pashto related to these issues on MAIL and other Afghan government social media platforms;
- Guidance on the identification of relevant stakeholders in the government and private sector in export markets for Afghan products to include Afghan embassy officials, foreign government representatives, and foreign private sector representatives and the organization of meetings with these stakeholders;
- Training for MAIL communications staff on best practices for government health and safety-specific emergency communications;
- Guidance on the development of a crisis communications plan for future events that can be utilized by MAIL

All production of communications products and organization of events should be conducted in conjunction with MAIL to ensure that the Ministry communications team develops the capacity to implement a crisis communications plan in the future without outside support.

Target Audiences:

The target audiences for communications events and products produced as a result of this guidance and capacity building support include Afghan citizens who could potentially consume mycotoxins, Afghan farmers and agribusinesses whose crops may be affected by mycotoxins, Afghan government counterparts at the national, provincial, and district level who should remain fully informed and able to communicate on any issues related to the potential negative impacts of mycotoxins in Afghan agricultural products, and the national governments and private sector stakeholders of export markets for Afghan agricultural products.

Delivery Schedule:

Within xx days from the receipt of this scope of work, Kansas State University identify a strategic communications advisor and draft a crisis communications plan for socialization and clearance with the communications staff of MAIL and the USAID Office of Agriculture (OAG) and the Development Outreach and Communications Office (DOC). Any communications events and products that results from this plan will be dependent upon the strategy MAIL has developed to ensure that Afghan agricultural products are safe for export and consumption.

KSU Response to Scope of Work – Communications on Mycotoxins

Background:

In December 2014, Afghanistan's Ministry of Agriculture, Irrigation and Livestock (MAIL), with support from the United States Agency for International Development (USAID), established a laboratory to evaluate Afghan agricultural products for contamination by mycotoxins, toxic metabolites produced by some fungi. USAID also partnered with MAIL to conduct a rapid baseline assessment of mycotoxins in Afghan agricultural products from July-December 2015. The rapid assessment found that mycotoxins in Afghan 20-50% of the nuts and raisins exceeded international standards and 15-33% of wheat samples contained mycotoxin levels high enough to be of public health concern. USAID together with MAIL, MITC, MoPH and Kansas State University hosted a conference of project participants, stakeholders and international external experts in New Delhi from 14-16 March 2016 to determine next steps.

USAID is requesting support from Kansas State University to provide strategic communications guidance and capacity building support for MAIL to effectively and accurately communicate to Afghan citizens, government counterparts, and the international community concerning the prevalence of mycotoxins in Afghan agricultural products, the dangers they pose, and the strategy MAIL has developed to ensure that Afghan agricultural products are safe for export and consumption.

Deliverables:

Kansas State University, working in conjunction with the USAID Office of Agriculture (OAG) and the Development Outreach and Communications Office (DOC), will arrange for a strategic communications advisory team to provide communications guidance and capacity building support for MAIL based upon strategies developed by MAIL to increase the safety of Afghan agricultural products for export and consumption. All of the information provided will be available in English. Portions of the information provided should be available in Dari, and Pashto, languages in which K-State lacks the expertise necessary to provide qualified translations both literally and in terms of the cultural context within the country. Areas for guidance and capacity building support may include but are not limited to:

1. Guidance on the development of a strategic communications plan based upon strategy developed by MAIL to increase the safety of Afghan agricultural products for export and consumption. This plan will include guidance on methodology and timelines for initially informing relevant audiences and for keeping these audiences informed as the implementation of MAIL's strategy progresses. This plan will include performance benchmarks that can be monitored and evaluated;
2. Guidance on monitoring of domestic and international traditional media and social media concerning mycotoxins and MAIL's ability to address the problem;

3. The polling of target audiences over the course of the implementation of the communications plan to track changes in opinion and understanding of relevant issues;
4. Guidance on the organization of press conferences, media roundtables, meetings and town halls at the national, provincial, and community level to inform and educate the general public, farmers, and agribusinesses on how to increase the safety of Afghan agricultural products that will be delivered and/or simultaneously translated into English, Dari, and Pashto;
5. Guidance on the advance drafting and translating of talking points, press releases, updates, and social media toolkits to be shared by MAIL and other Afghan government stakeholders;
6. Guidance on the production of radio and television PSAs to be broadcast across the country;
7. Guidance on the oversight of social media communications related to these issues on MAIL and other Afghan government social media platforms;
8. Guidance on the identification of relevant stakeholders in the government and private sector in export markets for Afghan products to include Afghan embassy officials, foreign government representatives, and foreign private sector representatives and the organization of meetings with these stakeholders;
9. Training for MAIL communications staff on best practices for government health- and safety-specific emergency communications;
10. Guidance on the development of a crisis communications plan for future events that can be utilized by MAIL.

All production of communications products and event organization will be conducted in conjunction with MAIL to ensure that the Ministry communications team develops the capacity to implement a crisis communications plan in the future without outside support.

Target Audiences:

The target audiences for communications events and products produced as a result of this guidance and capacity building support include Afghan citizens who could potentially consume mycotoxins, Afghan farmers and agribusinesses whose crops may be affected by mycotoxins, Afghan government counterparts at the national, provincial, and district level who should remain fully informed and able to communicate on any issues related to the potential negative impacts of mycotoxins in Afghan agricultural products, and the national governments and private sector stakeholders of export markets for Afghan agricultural products. The general approach to these audiences, type of information needed, and their respective health risks are outlined in the attached PowerPoint presentation.

Delivery Schedule:

Kansas State University has identified a strategic communications advisor (Mr. Jeffery Morris, VP for Communications and Marketing) and a technical adviser (Prof. John Leslie). Mr. Morris

and Prof. Leslie will develop a crisis communications and training plan for socialization and clearance with the communications staff of MAIL and the USAID Office of Agriculture (OAG) and the Development Outreach and Communications Office (DOC) within 90 days. Mr. Morris will utilize staff within his division at KSU, including experts in crisis communications, social media, press relations, writing (for the press and general audiences), and radio and television broadcasting as necessary to develop the plan. Implementation of communications events, products and training activities that result from this plan depend upon the strategy(ies) developed by MAIL to increase the safety of Afghan agricultural products for export and consumption. K-State staff will remain available to assist with plan implementation and training for up to 24 months from the start of the project.



Post-conference Workshop on Food Quality and Safety Creating a shared vision and partnership USAID Office of Agriculture (OAG)

DATE: July 16, 2016

LOCATION: U.S. Embassy, NOX building, Multi-Purpose Room A

OBJECTIVES:

- a) Review and discuss findings of “Rapid Assessment of Mycotoxins in Afghanistan’s Food Value Chains” and Action Plan that was prepared during March 2016 conference in New Delhi, India
- b) Discussion on Risk Communication and development of plan for action
- c) Discussion on Science, Technology, Information and Partnership (STIP) for agriculture

16 July 2016

Time	Topics	Presenter/ Facilitator
09:00 – 09:15	Welcome Introduction of the participants Review of Agenda	John Cardenas, Deputy Mission Director McDonald Homer OAG Acting Office Director
09:15 – 10:00	Summary of Mycotoxin Assessment <ul style="list-style-type: none">▪ John Leslie, Kansas State University	
10:00 – 10:15	Questions & Answers	
10:15 – 10:30	Tea Break	
10:30 – 11:30	New Delhi Action Plan Review <ul style="list-style-type: none">▪ John Leslie, Kansas State University▪ Amanullah Alamzai, World Bank▪ Jahid Ahady, MAIL▪ Dr. Hamid Formuli/Dr. Najibullah Safi, MoPH▪ Mohammad Asghar Anwari, MoCI▪ Tomio Shichiri, Food and Agriculture Organization	McDonald Homer
11:30 – 12:30	Risk Communication Work Groups	Andja Cosic, CBCMP II

Time	Topics	Presenter/ Facilitator
12:30-13:30	Lunch	
13:45 – 14:30	Risk Communication Plan/Work Group reports <ul style="list-style-type: none"> ▪ Summary from groups ▪ Overall summary/next steps 	Andja Cosic, CBCMP II
14:30 – 14:45	Tea Break	
14:45 – 15:15	Science, Technology, Innovation and Partnership (STIP)	McDonald Homer
15:30 – 16:00	Workshop Summation <ul style="list-style-type: none"> ▪ Closing Remarks ▪ Group photo 	

Post-New Delhi Conference meeting
Participants List

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Dr. Kanishka Turkistani	MoPH	Director of Environmental Health				
Dr. Najibullah Safi	MoPH	GD Preventive Medicine				
Dr. Shafiqullah Hemat	MoPH	Director of Health Promotion				
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Kikuchi Yukiko	JICA					
Nakahara Masataka	Jica					
Charles Richard Davy	UK AID					
Dr. Giancarlo Ferrari	FAO				Badge	FAO-CV00053
Dr. Latifi Muhebullah	FAO				Badge	FAO-NS00299
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Mo Salman	Biosecurity Engagement Program US Embassy	Coordinator				



Post-conference Workshop on Food Quality and Safety
Risk Communication Session



Ministry of Agriculture, Irrigation and Livestock			
Questions	Answers	Level of Risk/Priority	Deadline
What do we know about Mycotoxins? What are the public health risks and economic risks?	<i>(This should help to summarize information received/acquired during the workshop).</i>	High, medium or low priority	(When we should start /how long preparation is needed?)
Whom to ask?/How to double-check?	<i>(This should help participants to identify/remember relevant institutions and sources of information).</i>		
What are our objectives?/What we want to achieve communicating?	<i>(This should help participants to define objectives of the communication campaign/activities).</i>		
Whom we are talking to?/Who are our audiences?	<i>Primary audience</i>		
	<i>Other audiences</i>		
What are our messages?	<i>Priority Message</i>		
	<i>Supporting message 1</i>		
	<i>Supporting message 2</i>		
What are the challenges and risks we have to take into account?			
Which communications tools and channels we are going to use?			
How we will monitor our communication activities?			
How we will evaluate the impact?			



Post-conference Workshop on Food Quality and Safety
Risk Communication Session



Ministry of Public Health			
Questions	Answers	Level of Risk/Priority	Deadline
What do we know about Mycotoxins? What are the public health risks and economic risks?	<i>(This should help to summarize information received/acquired during the workshop).</i>	High, medium or low priority	(When we can start /how long preparation is needed?)
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Post-conference Workshop on Food Quality and Safety
Risk Communication Session



Ministry of Commerce and Industries			
Questions	Answers	Level of Risk/Priority	Deadline
What do we know about Mycotoxins? What are the public health risks and economic risks?	<i>(This should help to summarize information received/acquired during the workshop).</i>	High, medium or low priority	(When we can start /how long preparation is needed?)
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What are the challenges and risks we have to take into account?			
Which communications tools and channels we are going to use?			
How we will monitor our communication activities?			
How we will evaluate the impact?			

Mycotoxin Survey Overall Results

John F. Leslie

Department of Plant Pathology

Kansas State University

What are Mycotoxins?

- Natural toxic metabolites produced by fungi
- Problems known at least since Ancient Greece
- Six agriculturally most important mycotoxins:
 - Aflatoxins
 - Fumonisin
 - Deoxynivalenol and other trichothecenes, *e.g.*, T-2
 - Zearalenone
 - Ochratoxin
 - Ergot Alkaloids

Some also are potent carcinogens and mutagens

Several toxins are 3-5 orders of magnitude more toxic than fungicides that control them and break down more slowly

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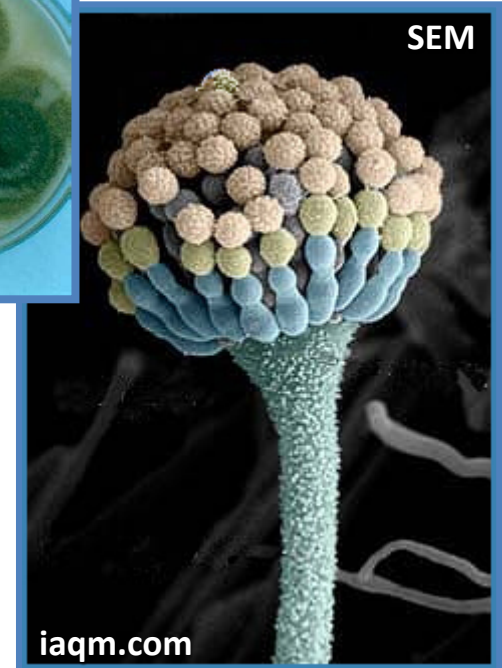
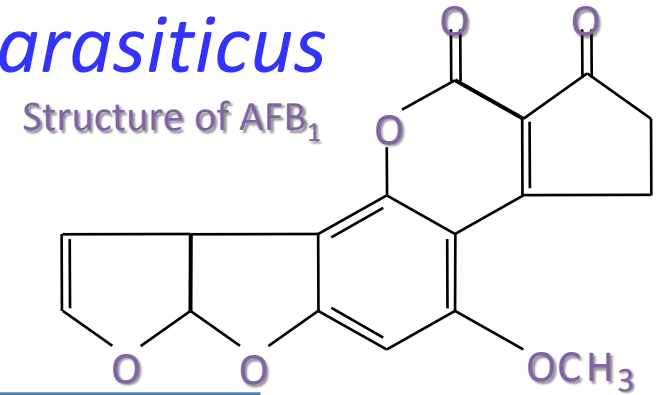
Several toxins are 3-5 orders of magnitude more toxic than fungicides that control them and break down more slowly

What Was Done?

- Afghanistan – MAIL and Deb Frey
 - Trained staff
 - Established and equipped a functional lab
 - Collected samples from across Afghanistan
 - Assayed raisins, nuts & wheat with Romer test kits
- Italy – ISPA
 - UPLC and LC-MS assays for trichothecenes in wheat
 - Mycological analyses of flour
- Austria – BOKU
 - Quadripole MS assay for 650 different metabolites
- USA – K-State and Univ. Nebraska-Lincoln
 - Assay nuts and wheat with Romer test kits
 - Test kits from Vicam and Neogen
 - Mycological analyses of flour
 - Synthesize results
 - Test reliability of test kits

Aflatoxins

Aspergillus flavus / *A. parasiticus*



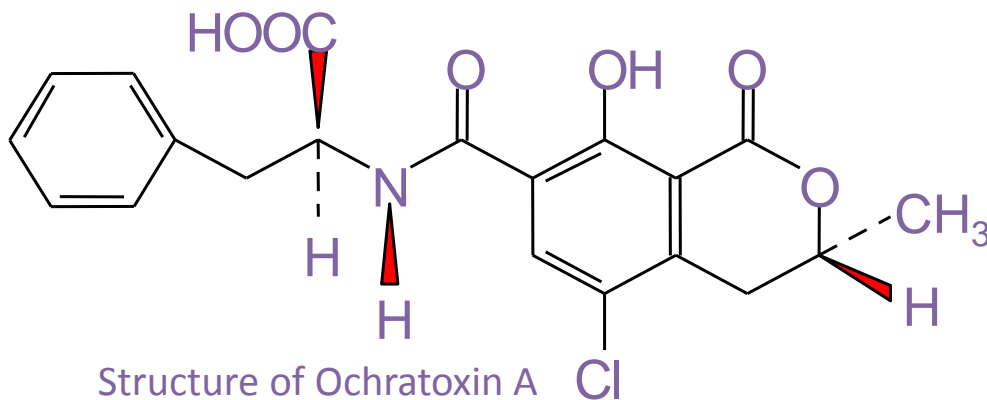
Liver failure
Liver cancer
Growth stunting
Immune deficiency or
suppression
Grains – especially maize
Peanuts
Tree nuts & raisins

Ochratoxins

Aspergillus ochraceus



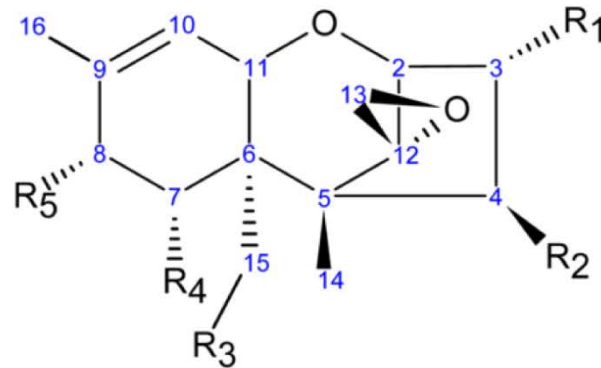
Kidney failure
Cacao
Tree nuts
Grapes (raisins
& wine)
Coffee
Wheat



Trichothecenes

- Two classes – A & B, strains make only one type
- Both inhibit protein synthesis
- Most common in grains
- Type A – very toxic – T-2, HT-2 & DAS
 - US Select agent list
 - Purportedly used for biological warfare
- Type B – not as toxic – DON, NIV
 - More widespread, especially on wheat
- Can be taken up through skin or intestinal mucosa
- Cause vomiting, diarrhea, & immune suppression

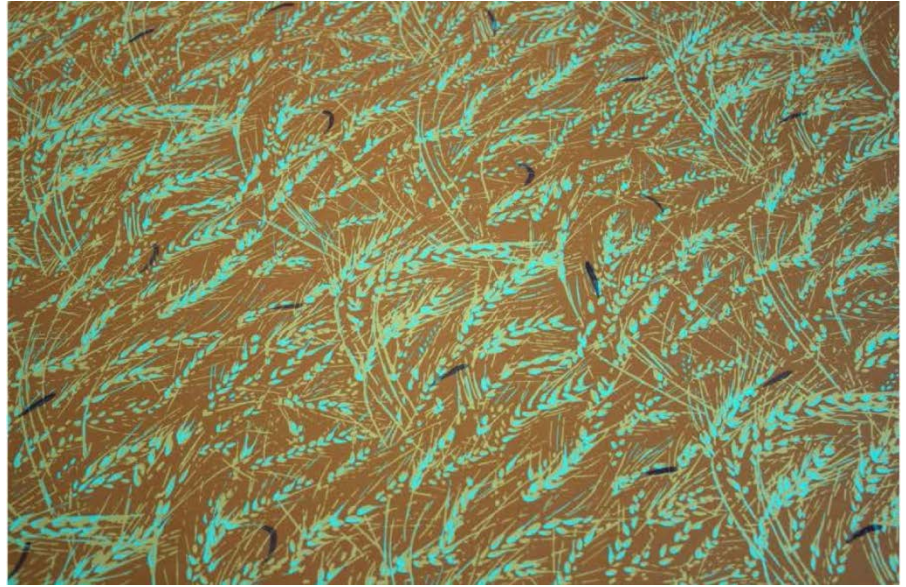
Toxin	R1	R2	R3	R4	R5
DON	-OH	-H	-OH	-OH	=O
3-ADON	-OAc	-H	-OH	-OH	=O
15-ADON	-OH	-H	-OAc	-OH	=O
NIV	-OH	-OH	-OH	-OH	=O
T-2	-OH	-OAc	-OAc	-H	-Olsoval
HT-2	-OH	-OH	-OAc	-H	-Olsoval
4,15-DAS	-OH	-OAc	-OAc	-H	-H



T-2 – Killed 1000s in Russia after WWII
DON – Becoming very widespread in US and Europe, especially where wheat and maize are grown
DON is changing the economic landscape of the US Great Plains
Fusarium is the main producer on grains, but other fungi and some plants also synthesize

Ergot Alkaloids

- Small Grains – Wheat, rye, barley & oats
- An unexpected finding by Austrian group
- Not highly regulated (animal feed only)
- In small doses – hallucinations (LSD)
- In other cases – neuropathy and gangrene
- Gnostics and ancient Greeks may have used them to help people have visions
- Controlled by sorting ergot bodies from the grain before processing



Some Ergot Epidemics

-600 – Assyria

857 – Germany

945 – France

1093 – France

1692 – USA

1926 – Russia

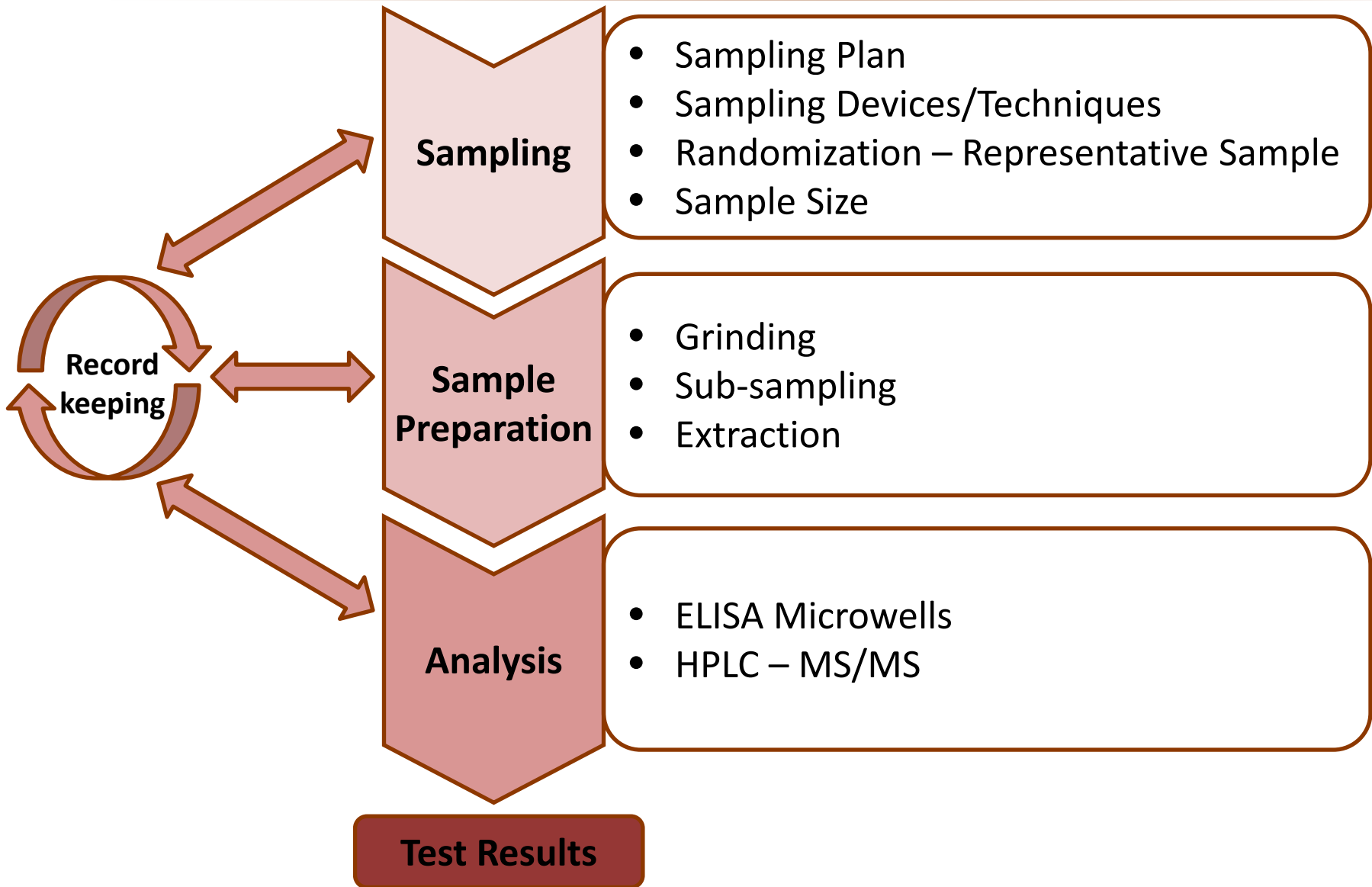
1929 – Ireland

1953 – France

1958 – India

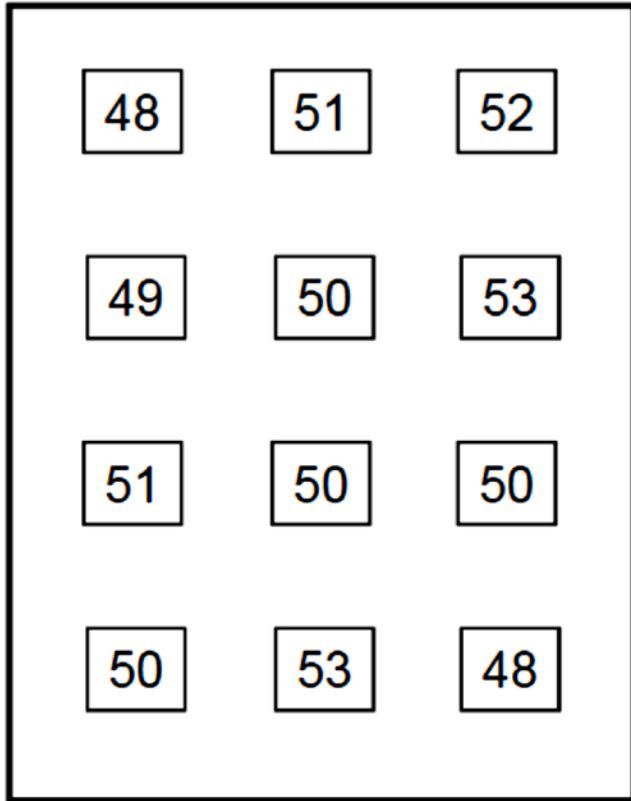
1973 – Ethiopia

Mycotoxin Analysis – Main Steps

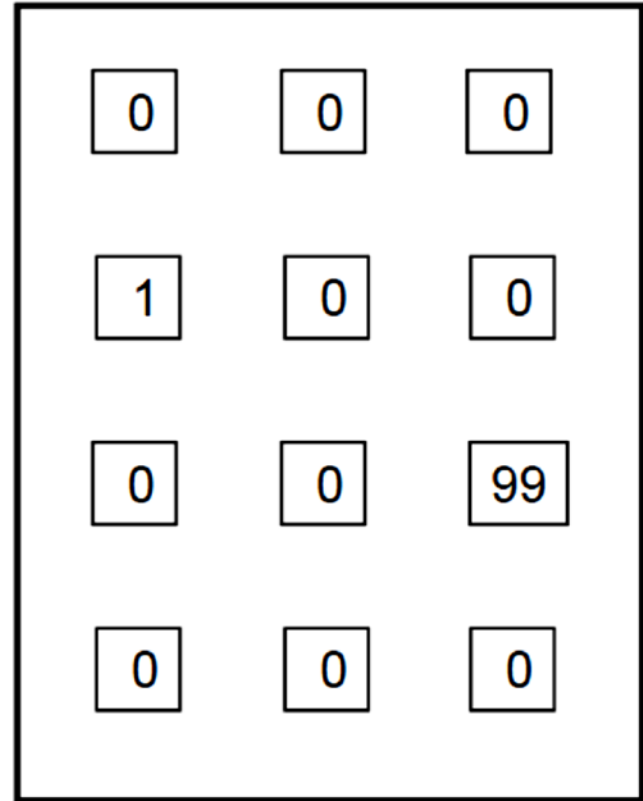




Sampling



Typical Protein Distribution



Typical Mycotoxin Distribution

Impact of Splitting Before Grinding

Mycotoxin ($\mu\text{g}/\text{kg}$)	Commodity	Batch 1	Batch 2		
		Afghanistan	Austria	USA (UNL)	USA (KSU)
Aflatoxin	Wheat-68	5	< LOD	< LOD	< LOD
	Wheat-110	10	< LOD	< LOD	< LOD
	Walnut-551	25	< LOD	< LOD	
	Pistachio-624	< LOD	15	95	
Deoxynivalenol	Wheat-3	3500	< LOD	< LOD	< LOD
	Wheat-14	1290	< LOD	< LOD	< LOD
Ochratoxin	Raisin-296	13	< LOD		
	Raisin-302	< LOD	10		

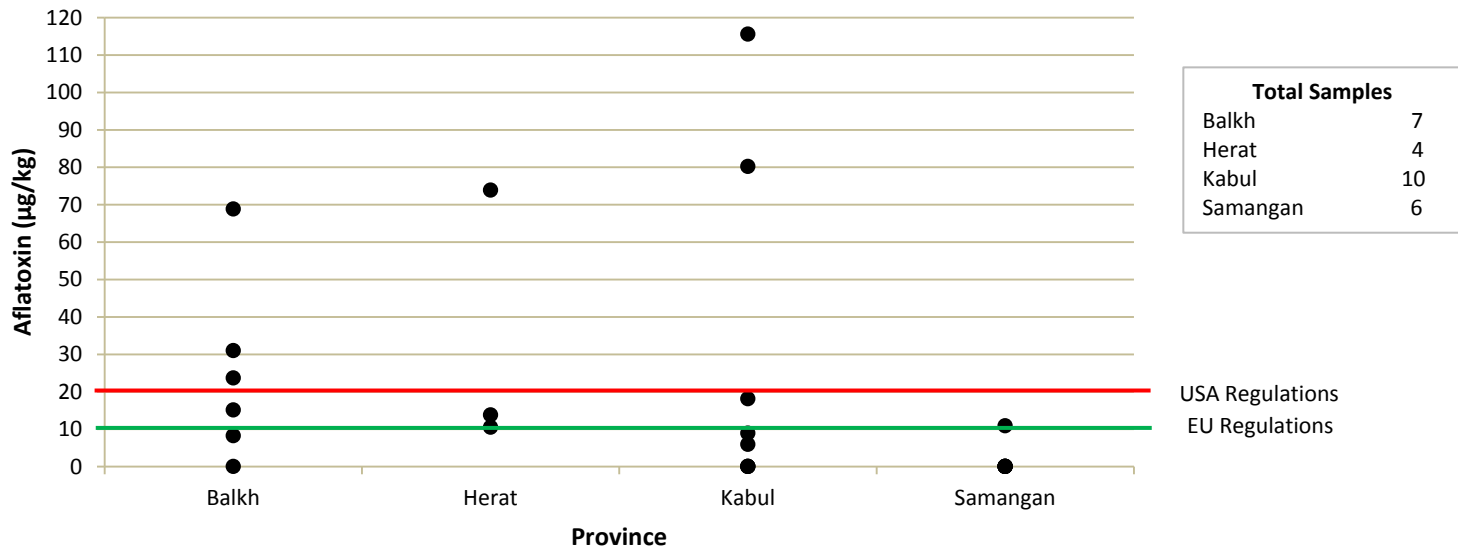
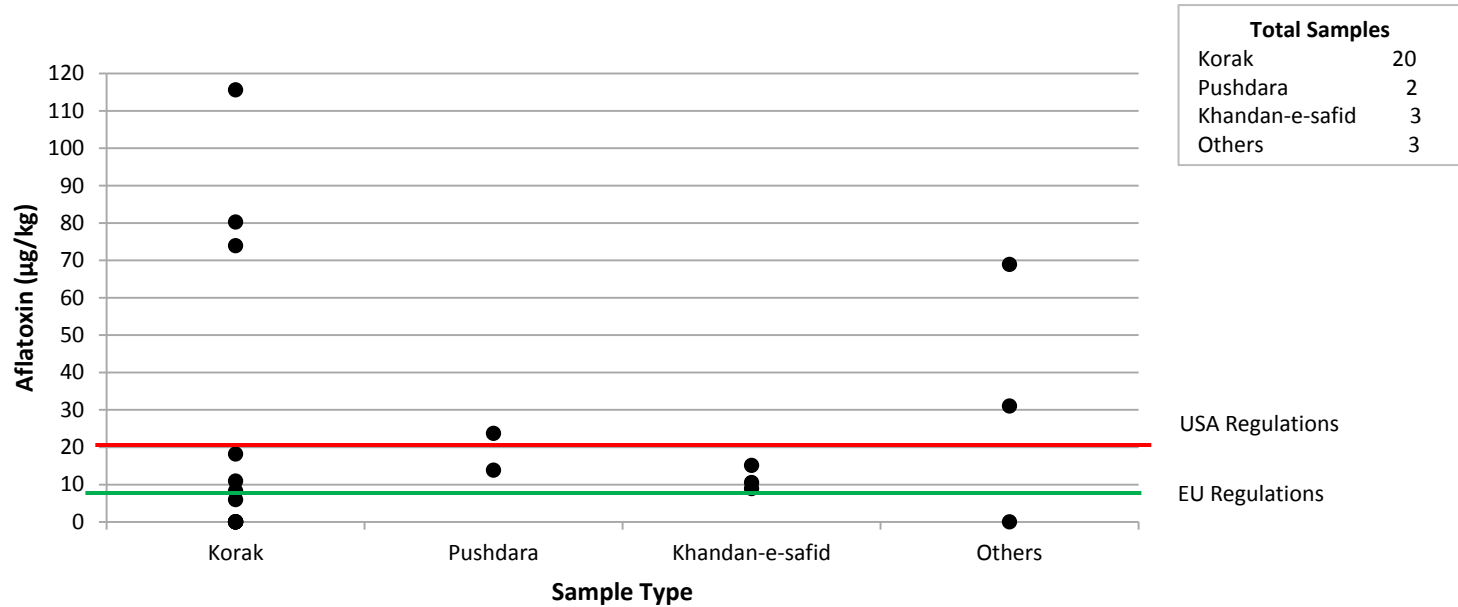
Test Kit Performance

- Romer and Neogen kits for aflatoxin, ochratoxin and deoxynivalenol (DON or vomitoxin) were reliable for all tested substrates
- Vicam kits for ochratoxin and DON were reliable for wheat, but aflatoxin kit was not
- Romer test for T-2 toxin was erratic
- Neogen and Romer tests for T-2/HT-2 toxins gave many (Romer) and exclusively (Neogen) false positives in wheat

Nuts – Results

- Aflatoxin
 - Almonds – 15/81 at export limiting level
 - Pistachios – 19/40 at export limiting level
 - Walnuts – 8/25 at export limiting level
- Ochratoxin
 - Almonds – no contamination detected
 - Pistachios – 2/40 at export limiting levels
 - Walnuts – no contamination detected

Pistachios – Aflatoxins



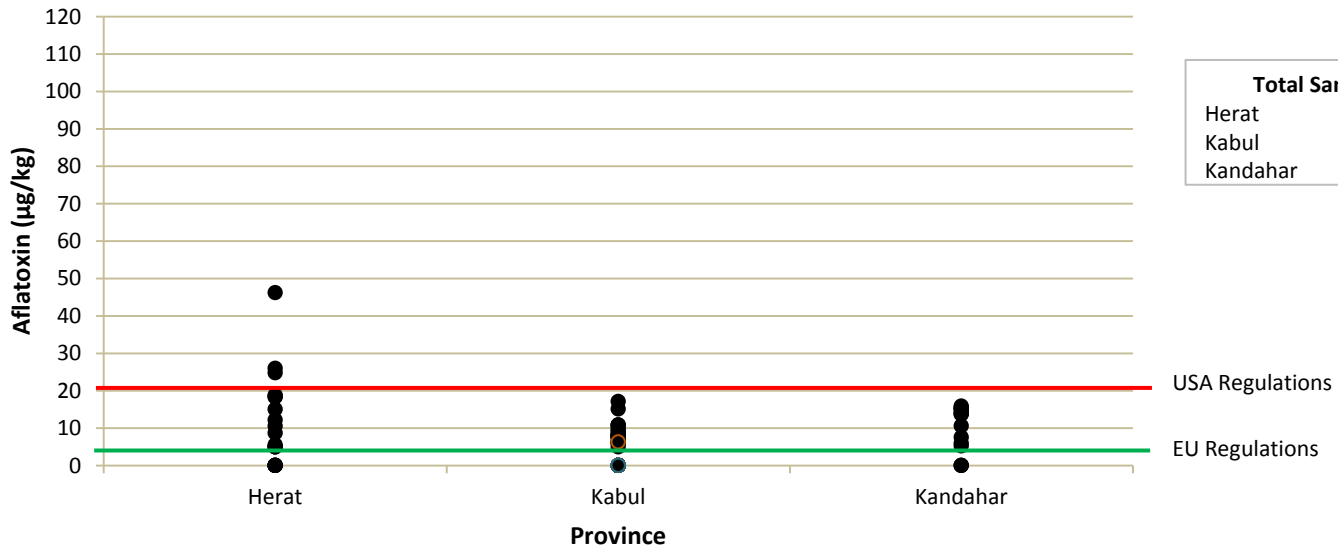
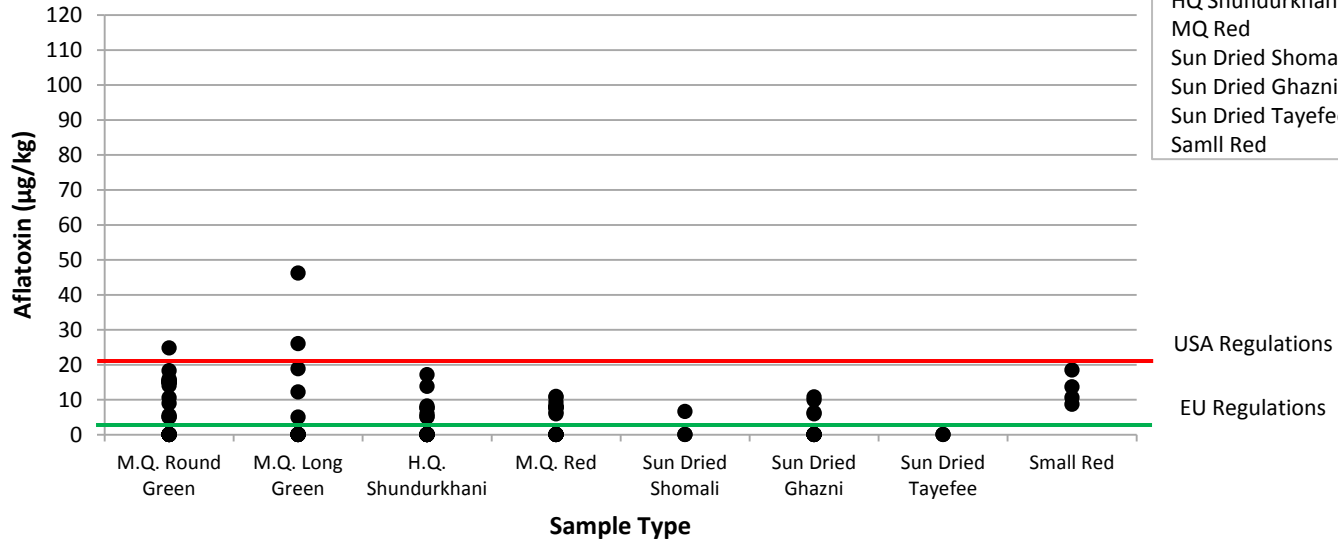
Austrian Screen – Nuts

<i>Fusarium</i>	<i>Alternaria</i>	<i>Aspergillus</i>	<i>Penicillium</i>
Butenolide	Alternariol	Cyclopiazonic Acid	Andrastin A
	Alternariol methyl ether	Aflatoxin	Andrastin B
Epiequisetin	Altersetin	Asperfuran	Agroclavine
Equisetin	Infectopyron	Kojic acid	Chanoclavin
Fusaric acid	Macrosporin	Malformin A	Epoxyagroclavin
HT-2 toxin	Tentoxin	Malformin A2	Festuclavine
T-2 toxin	Tenuazonic acid	Malformin C	Mycophenolic acid
Zearalenone			Mycophenolic acid IV
α -Zearalenol		Nigragillin	
β -Zearalenol		3-Nitropropionic acid	Penitrem A
		Ochratoxin	
		Paspalin	

Raisins – Results

- Aflatoxins could limit exports in 43/89 samples
- Ochratoxin could limit exports in 25/80 samples
- Raisin type and drying method can be important
- Afghanistan and Austria results are discordant
- Choice of country to export to may depend on level of contamination

Raisins – Aflatoxins



Austrian Screen – Raisins

<i>Fusarium</i>	<i>Alternaria</i>	<i>Aspergillus</i>	<i>Penicillium</i>
Fumonisin	Alternariol	Aflatoxin	Andrastin A
	Alternariol methyl ether	Aurasperon B	Andrastin B
	Altersetin	Aurasperon C	Andrastin C
	Altertoxin-I	Aurasperon G	Chanoclavin
	Macrosporin	Fonsecin	Festoclavine
	Tentoxin	Malformin A	Mycophenolic acid
	Tenuazonic acid	Malformin A2	Mycophenolic acid IV
		Malformin C	Penitrem A
		Nigragillin	Quinolactacin A
		Ochratoxin	
		Pyranonigrin	

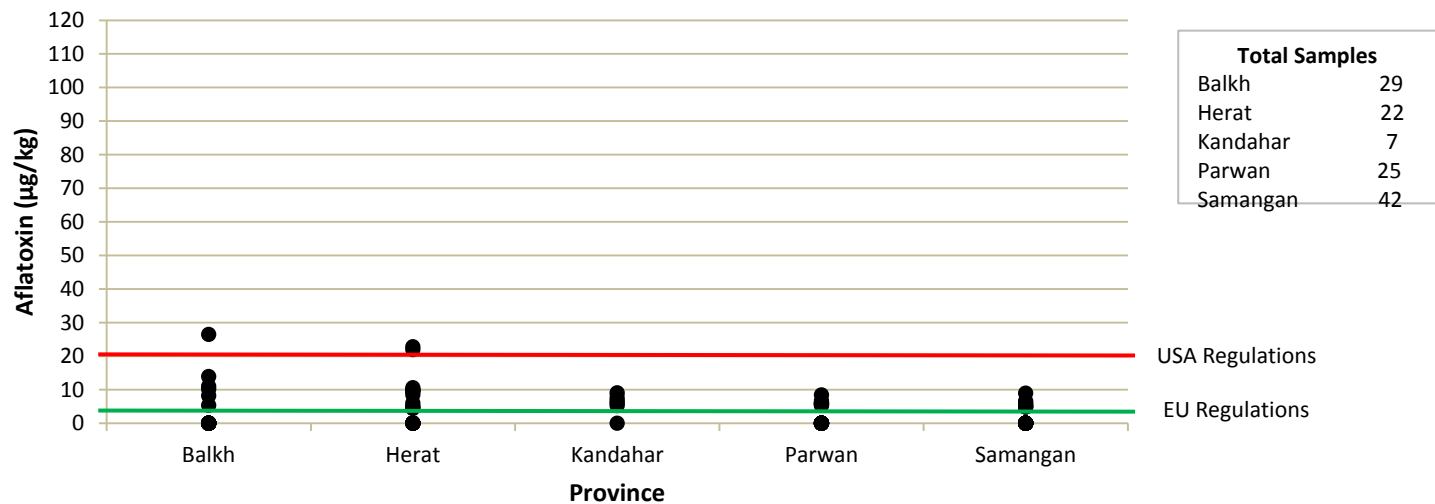
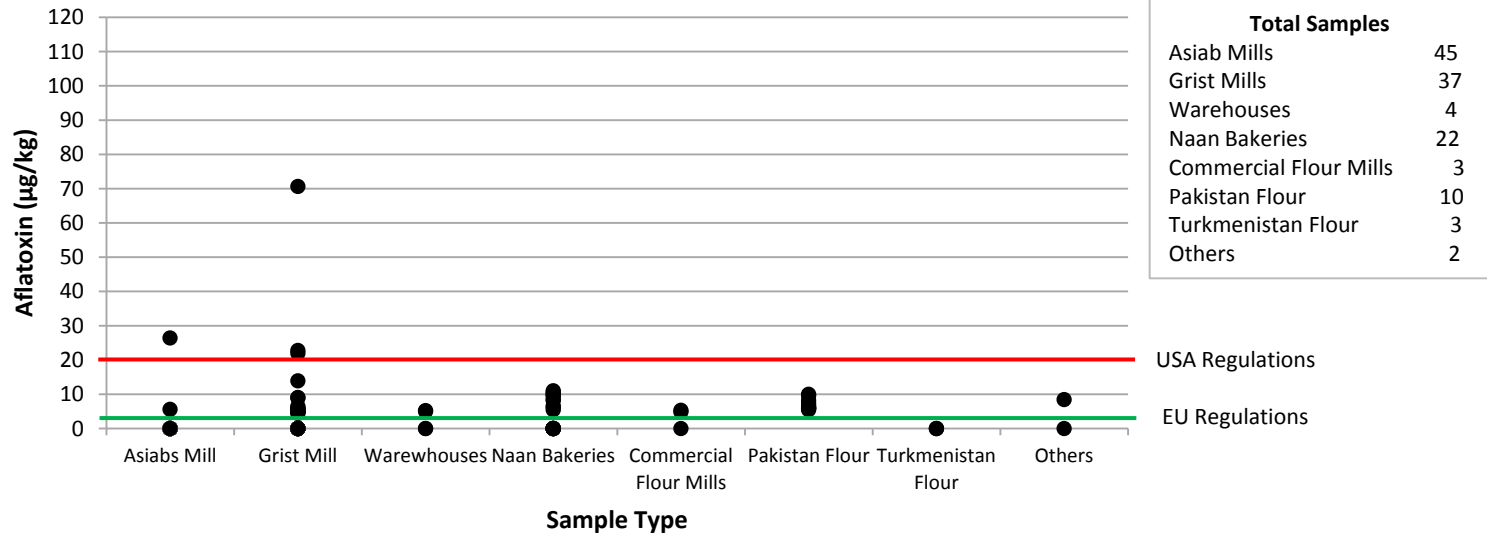
Wheat – Results

- International standards may be too high for Afghanistan safety because of the large amount of wheat consumed daily (500 g/person/day)
- Aflatoxins – detectable in 23/151 samples
 - Not a field contaminant of wheat
 - Contamination likely occurs in storage
- DON – 3/185 above international guidelines
 - Not a major problem, but exists
 - Weather and storage dependent
- Ochratoxin – detectable in 36/181
 - Common problem in northern Europe
 - Needs attention
 - May carry over to meat
- T-2 and HT-2 – Not reliably detected
- Ergot – detectable 51/151
 - High incidence
 - Easily remedied by cleaning grain



Wheat – Aflatoxin

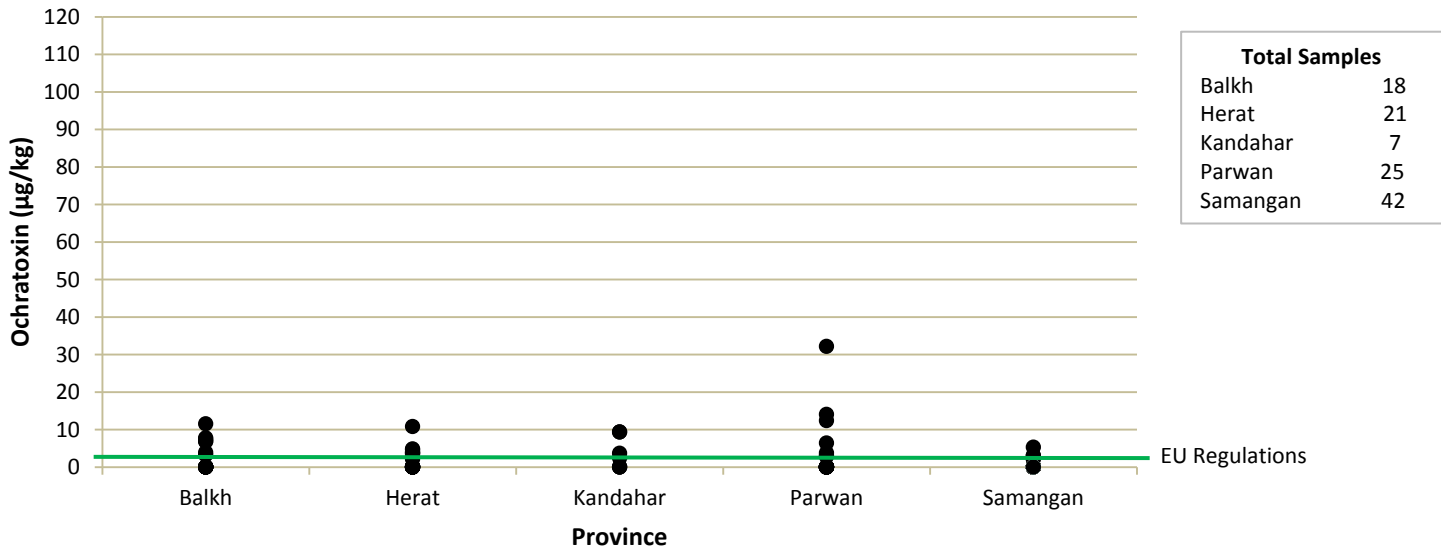
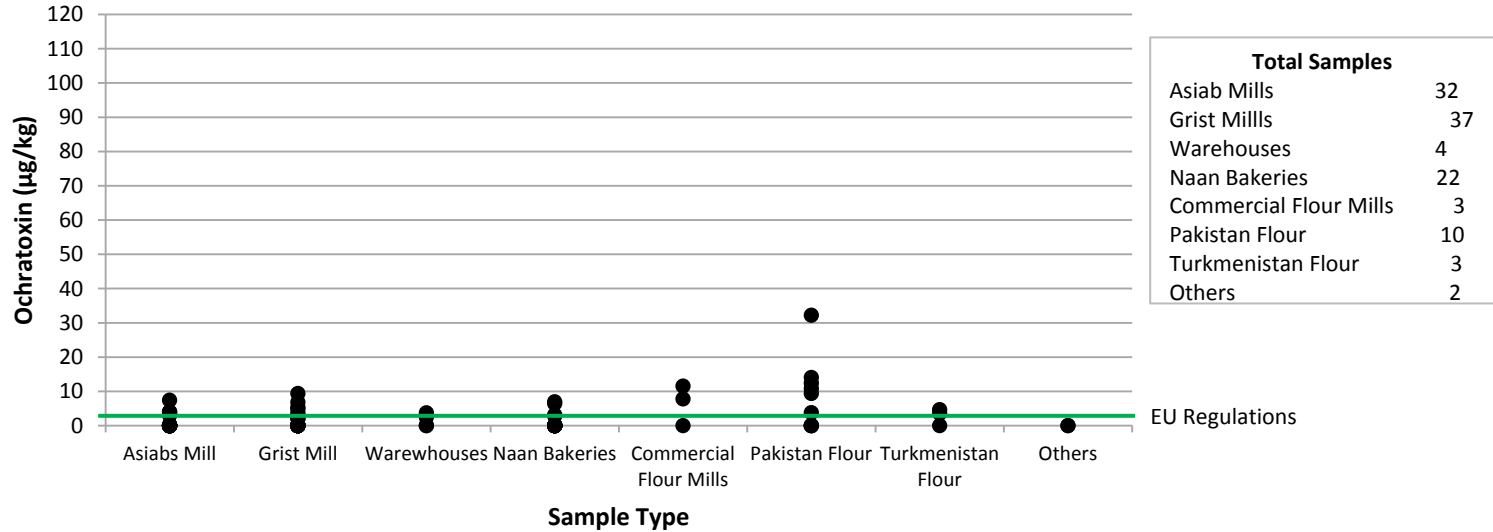
Afghan Diet Safety Limit – 0.14 $\mu\text{g}/\text{kg}$





Wheat – Ochratoxin

Afghan Diet Safety Limit – 0.7-9.8 $\mu\text{g}/\text{kg}$



Austrian Screen – Wheat

<i>Fusarium</i>	<i>Alternaria</i>	<i>Aspergillus</i>	<i>Penicillium</i>	<i>Claviceps</i>
Beauvericin	Alternariol	Aflatoxin	Agroclavine	Ergocristine
Enniatin A	Alternariol methyl ether	Averantin	Chanoclavine	Ergocristinine
Enniatin A ₁	Altersetin	Averufin	Citrinin	Ergometrine
Enniatin B	Altersolanol	Cycloaspeptide A	Chrysogine	Ergometrinine
Enniatin B ₁	Altertoxin-I	Kojic acid	Elymoclavine	Ergosin
Epiequisetin	Macrosporin	Methoxysterigm atocystin	Mycophenolic acid	Ergosinin
Equisetin	Tentoxin	3-Nitropropionic acid	Questiomycin A	Ergotamine
HT-2 toxin	Tenuazonic acid	Norsolorinic acid	Quinolactacin A	Ergotaminine
T-2 toxin		Ochratoxin	Secalonic acid D	
Zearalenone		Sterigmatocystin		

Conclusions

- The test kit used can affect the results
- Sampling procedures are critical
- Pre- and Post-harvest processes both matter
- Nuts and aflatoxins – Pistachios > Walnuts > Almonds
- Raisins – Aflatoxins > Ochratoxin
 - Type of raisin and drying method important
- Wheat
 - Need to evaluate safety levels for Afghan diets
 - Aflatoxin is a storage issue
 - *Fusarium* toxins (T-2, HT-2, DON & Zearalenone) are minimal
 - Citrinin + ochratoxin could enhance kidney problems
 - Ergot – high frequency, but relatively easy to fix



Special thanks to all of our collaborators in Afghanistan, Austria, Nebraska, Kansas (and my wife!).

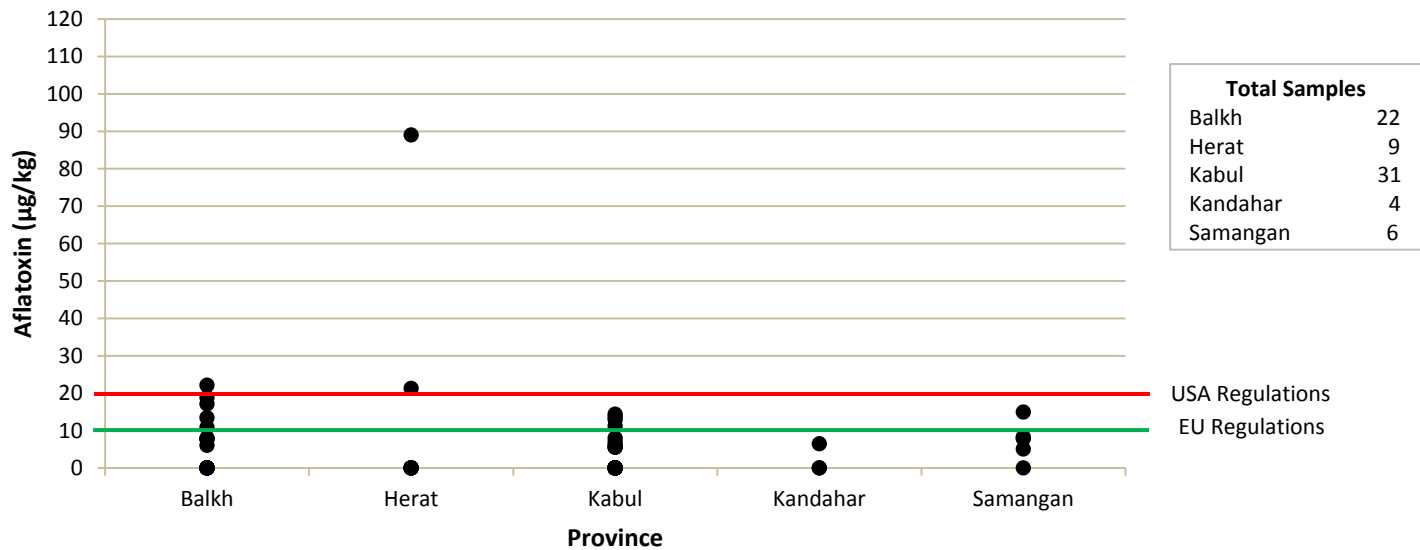
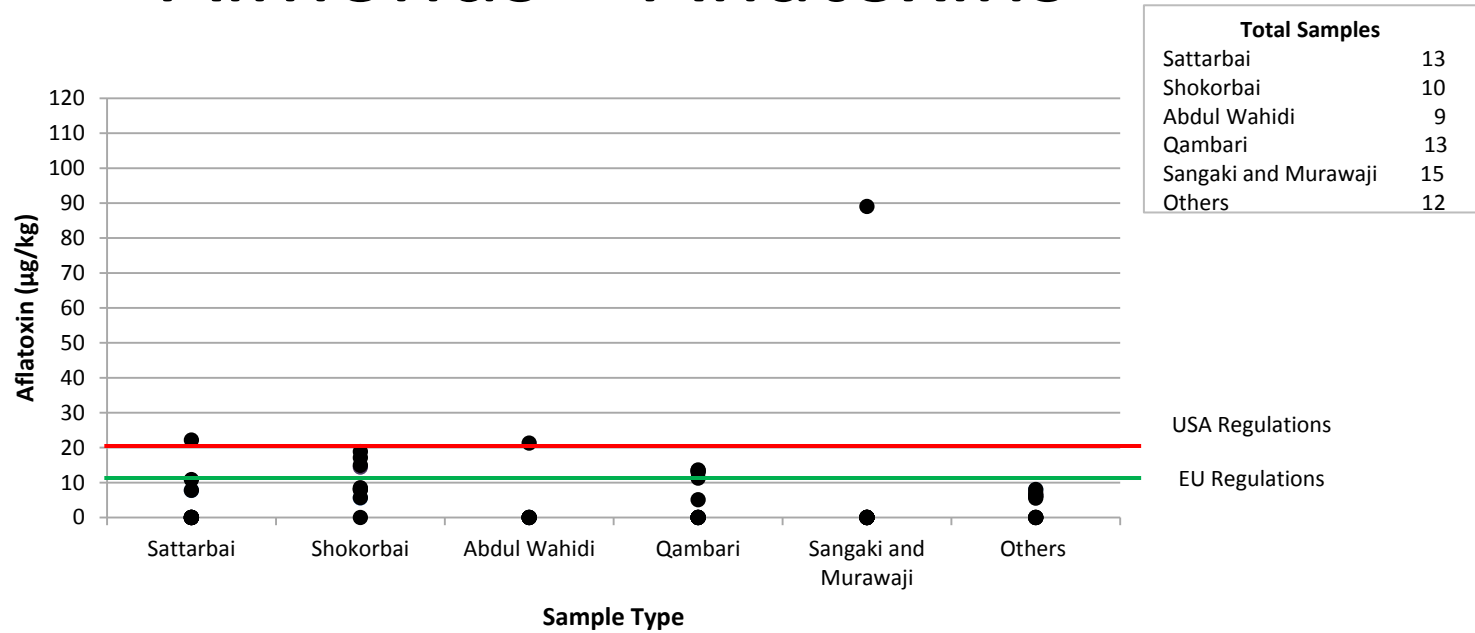
Questions?

“Where waters are
murky, crocodiles lurk!”

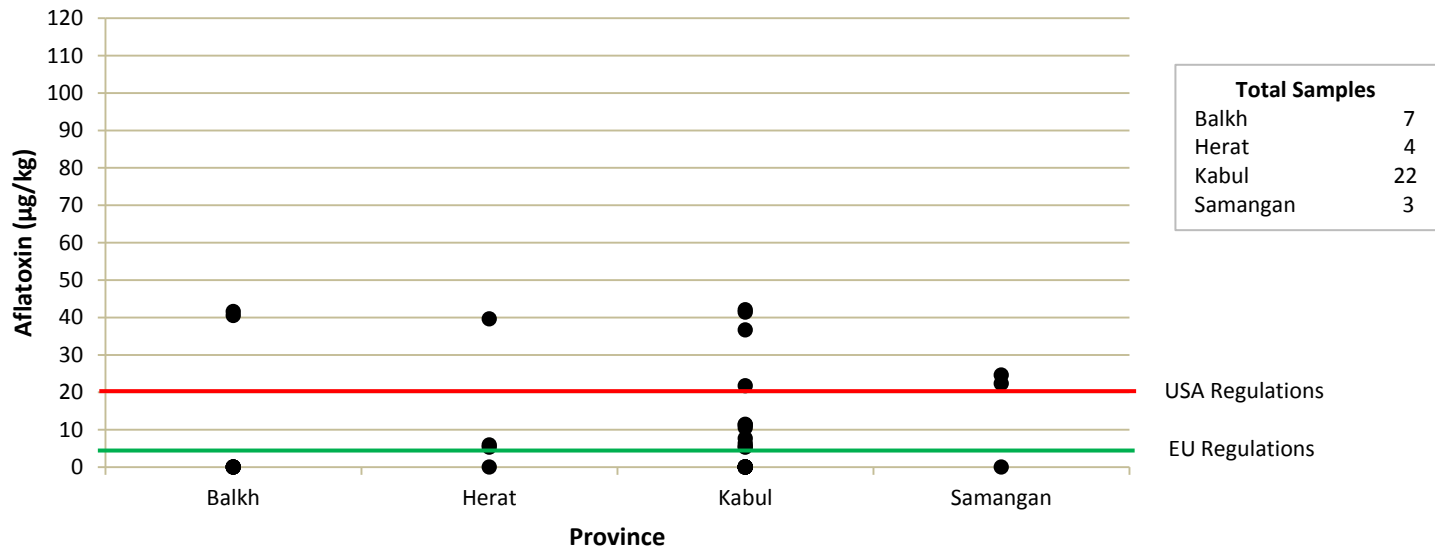
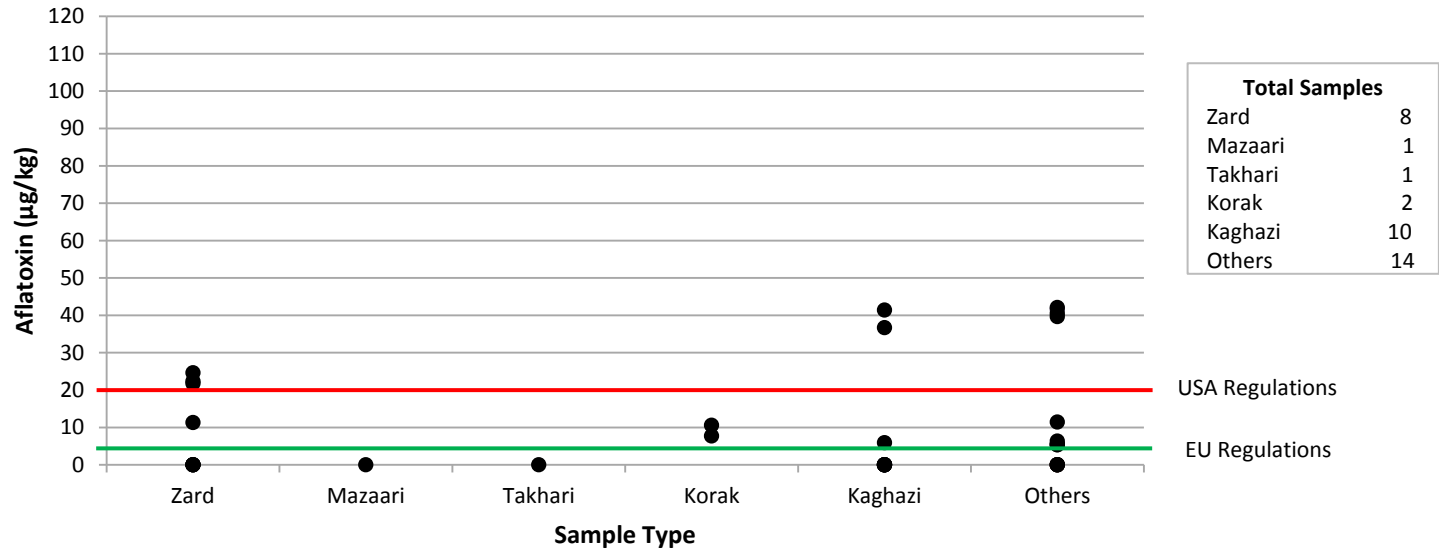
- Old African saying



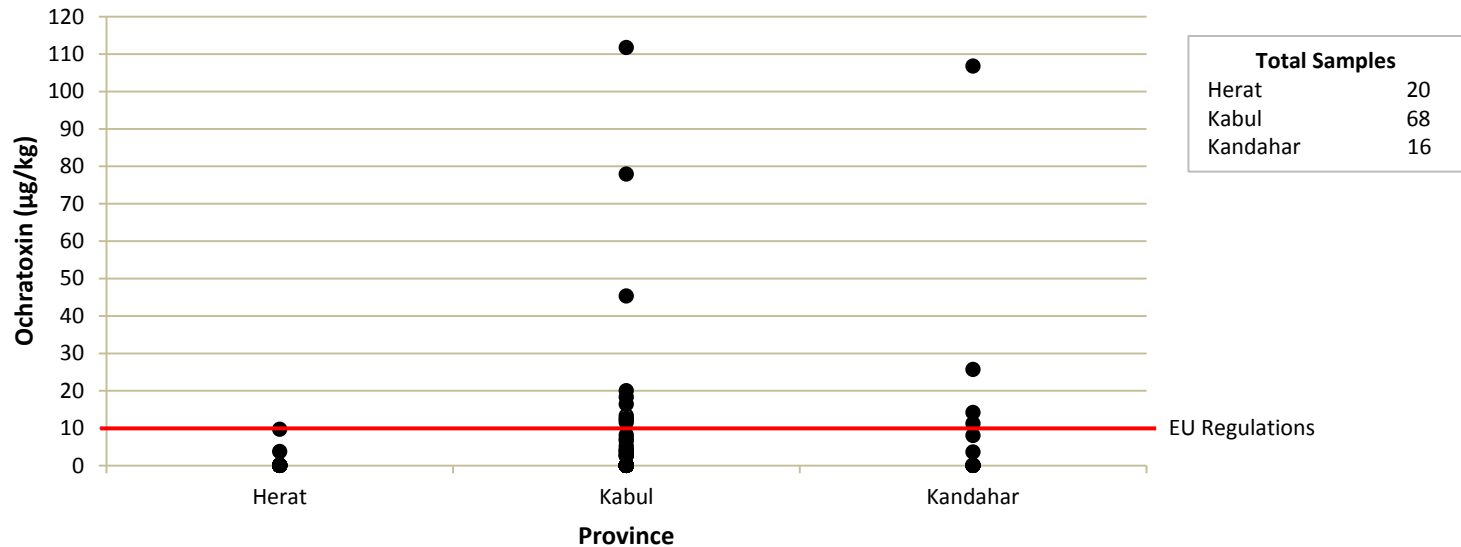
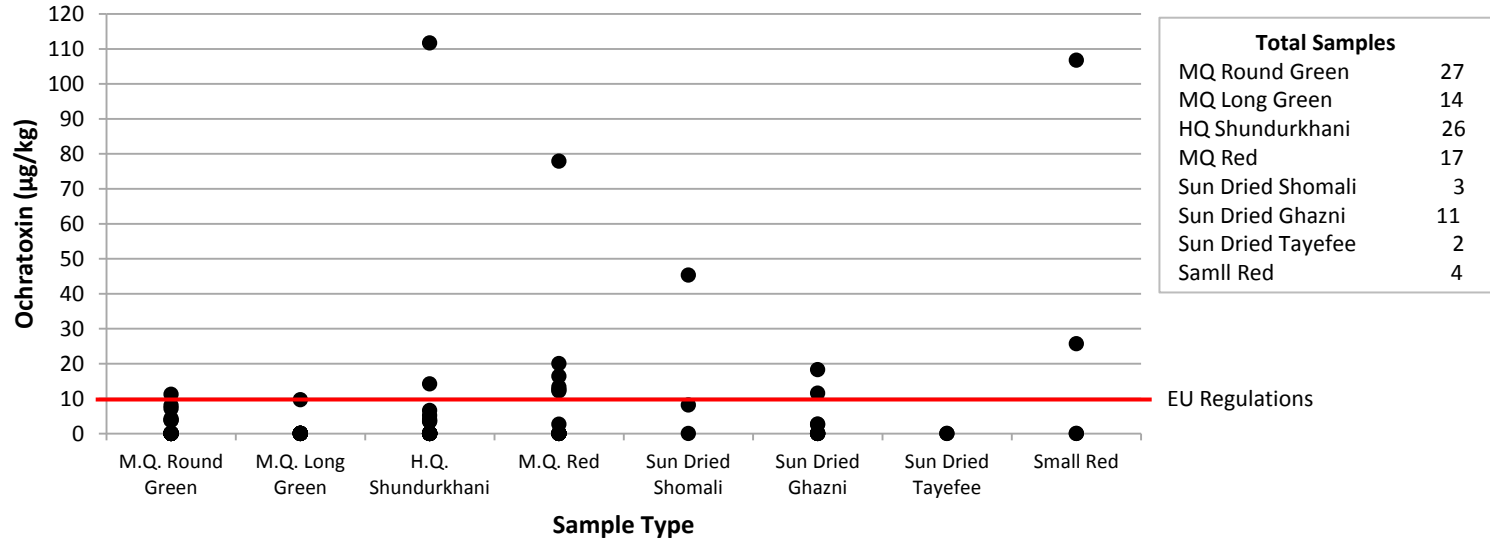
Almonds – Aflatoxins



Walnuts – Aflatoxins



Raisins – Ochratoxin



USAID – KSU Afghanistan Mycotoxin Meeting New Delhi 13-16 March 2016



Major Meeting Goals

- Presentations on topics of relevance considering previous experience/results and potential future projects
- Field trips to visit:
 - Indian Food Safety & Standards Authority
 - Airport Cargo Section @ Indira Gandhi Int. airport
 - Commercial food testing laboratory
- Nominal Group discussions based on project outcomes and projected future activities

Presentations

- Why the evaluation was requested
- What the lab did (and associated capacity building)
- Results of the study – good, bad & ugly
- Implications of mycotoxin contamination
 - Trade
 - Human health
 - Laboratory diagnostics
 - Communication of information to non-scientists
 - Means of control/biocontrol
- Place of mycotoxins in the larger food safety picture

Nominal Group Questions

- Identify capacity building required for mycotoxin surveillance
- Identify data needed for assessment of mycotoxins
- Identify ways to ensure results from Afghanistan are credible
- Goals for inter-ministry collaboration on mycotoxins
- Mycotoxin regulations needed in Afghanistan
- Cultural barriers to reducing mycotoxin exposure
- Benefits resulting from lower exposure to mycotoxins
- Who needs information on mycotoxins & how to deliver it?
- When/where should mycotoxin screening occur?
- Short/long-term priorities for mycotoxin work in Afghanistan

Conclusions – Awareness

- All tested products had some mycotoxin contamination
- Nuts/raisins and wheat have different needs & audiences
- Different groups of people have different needs
- Want to avoid unnecessary alarmism
- MAIL, MoPH & MITC need to work together to spread a common message
- Training at all levels and at different depths needed
- Differential market sensitivities to contamination
- Social media awareness tools, e.g. SWABO
- Focus on raising awareness without raising alarm



Conclusions – Capacity

- Research and regulatory needs are different
- Private labs may be preferable to government labs for regulatory purposes
- Human capacity requires long and short term training
- Physical capacity requires lab with essential equipment and functional infrastructure
- Expensive equipment not needed until someone can use it
- Need to work with universities as well as ministries
- Corruption can limit lab credibility if “no” is an unacceptable answer
- Laboratory accreditation is important by recognized authority



Conclusions - Medical

- No information from biomarkers currently available
- Data should be collected along with dietary information
- High wheat diet may lead to guidelines that differ from those in international settings
- Risks from acute and chronic exposures to be assessed
- Impacts of unregulated compounds may be important



Potential Action Items

- Identify alternative markets for some exports
- Determine degree of ergot contamination; develop sorter to remove ergot bodies
- Develop regulations for imported foods/feeds
- Develop HACCP and GAP guidelines for value chains
- Identify stakeholders, donors and leaders for future efforts
- Create Food Safety Authority and inter-ministerial working group
- Identify common ground for research with other countries in this region
- Continue research level survey to get better baseline data





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FROM THE AMERICAN PEOPLE

**Capacity Building and Change Management Program II
(CBCMP-II)**



**COMMUNICATION IN THE CONTEXT OF FOOD SAFETY
- RISKS AND OPPORTUNITIES -**

Kabul, July 16, 2016

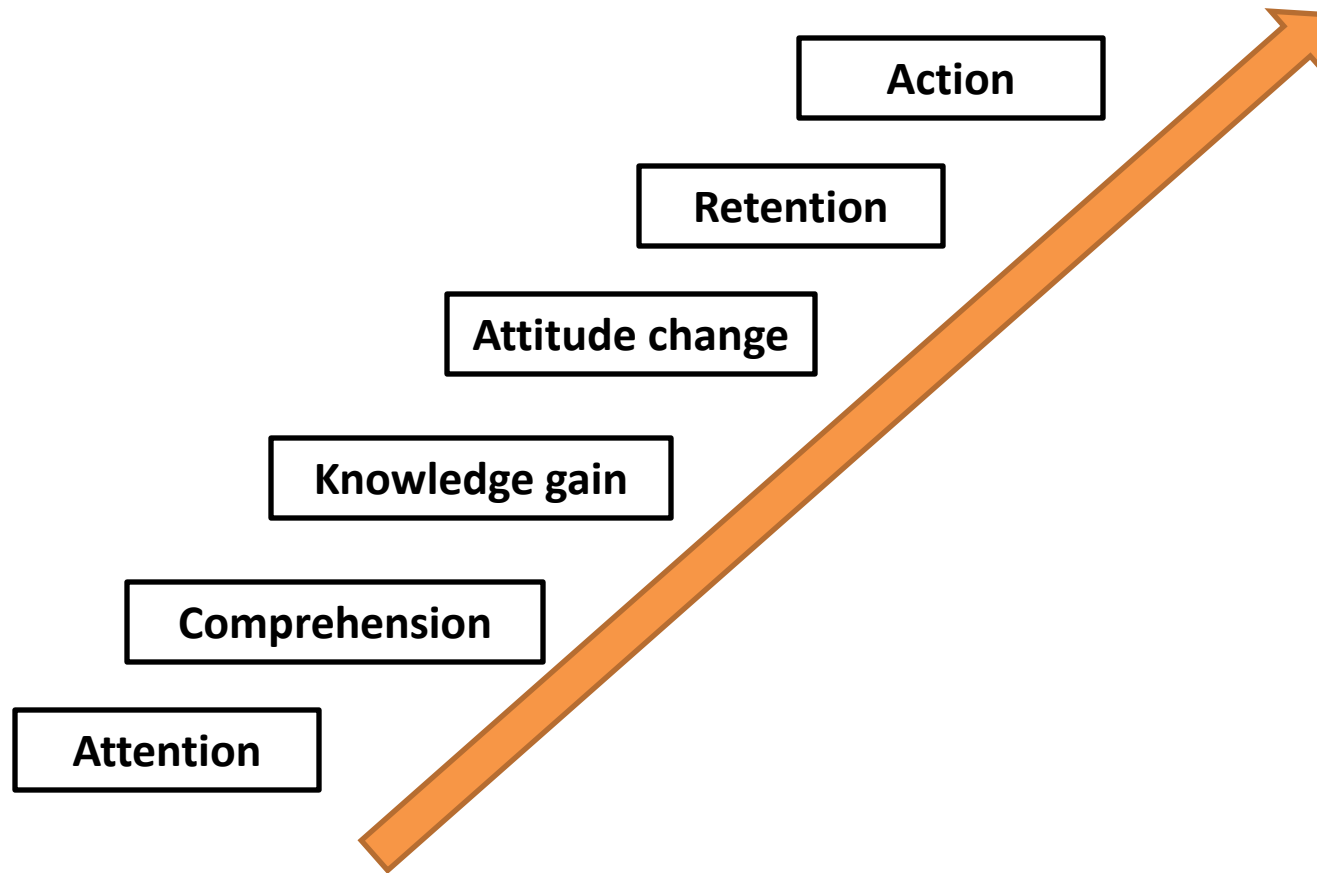
By Andja Cosic,
CBCMP-II Communications Director

Strengthening human and institutional capacity of the Ministry of Agriculture, Irrigation and Livestock to better serve Afghan farmers and herders





WHY DO WE COMMUNICATE ?



Strengthening human and institutional capacity of the Ministry of Agriculture, Irrigation and Livestock to better serve Afghan farmers and herders



WHAT THE COMMUNICATION IS?



Strengthening human and institutional capacity of the Ministry of Agriculture, Irrigation and Livestock to better serve Afghan farmers and herders



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Capacity Building and Change Management Program II (CBCMP-II)



FOOD SAFETY RISK COMMUNICATION

Food safety risk communication is important to protecting public, animal, plant and environmental health, and people's quality of life, including socio-economic factors such as livelihoods.

The goals of food safety risk communication are to enable people to protect their health by providing information that enables people to make informed food safety risk decisions, to facilitate dialogue and understanding between all interested stakeholders.

FAO and WHO definitions, Handbook on Risk Communication Applied to Food Safety

Strengthening human and institutional capacity of the Ministry of Agriculture, Irrigation and Livestock to better serve Afghan farmers and herders





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Capacity Building and Change Management Program II (CBCMP-II)



WHY AFGHANISTAN'S GOVERNMENT NEEDS TO COMMUNICATE ABOUT MYCOTOXINS

Presence of mycotoxins in food has harmful effects on the health of Afghan citizens;

Presence of mycotoxins negatively impacts export of Afghanistan's products, and therefore reduces income of Afghan farmers and their families;

With good communication and education of all relevant stakeholders, and Afghan public, many negative effects can be prevented.

Problem is fixable! Good practices already exist! (It is not happening only in Afghanistan, it is estimated that 25% of the world's crops are susceptible to some variety of mycotoxins, so let us see how other countries fixed their problems).

Strengthening human and institutional capacity of the Ministry of Agriculture, Irrigation and Livestock to better serve Afghan farmers and herders





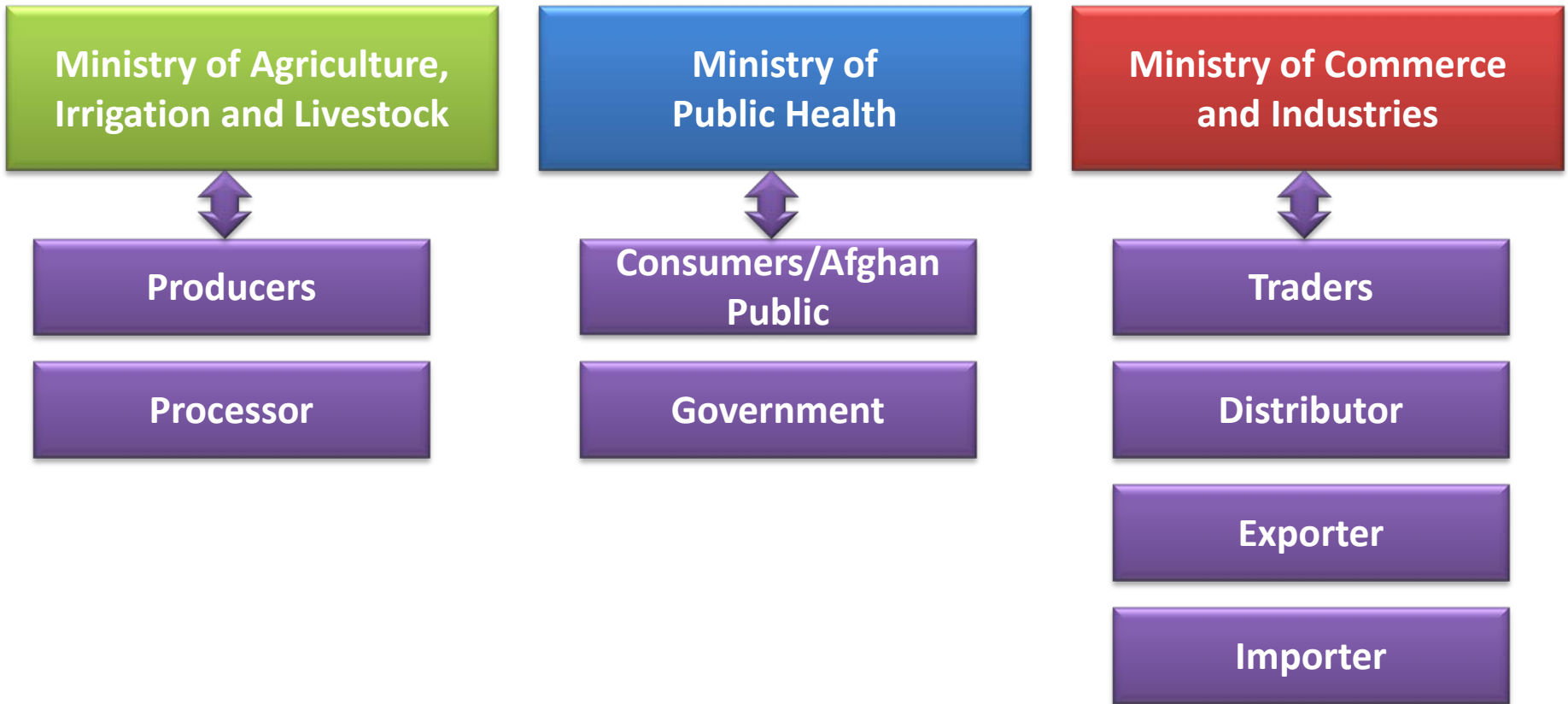
STAKEHOLDERS/TARGET AUDIENCES

	Producer	Trader	Processor	Distributor	Exporter	Importer	Government	Consumer
Technical Experts							✓	
Highly Literate	✓	✓	✓	✓	✓	✓	✓	✓
Average Literate	✓	✓	✓	✓	✓	✓	✓	✓
Nominally Literate	✓	✓	✓					✓
Illiterate	✓							✓

By Jeff Morris, Kansas State University



PRIMARY AUDIENCES BASED ON RESPONSIBILITIES



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WHY IT IS IMPORTANT TO INCLUDE STAKEHOLDERS?

Dialogue with stakeholders helps communicators to:

- Identify gaps in knowledge about the food safety risks that are under consideration.
- Understand stakeholders' risk perceptions and concerns.
- Identify potential communication barriers and the preferred and most appropriate information sources and channels of communication.
- Identify and address any *unintended consequences of the communication*.
- Generate more ideas.
- Expose concerns not otherwise recognized.
- Include different perspectives.
- Potentially create buy-in and builds broad support for the communication effort.
- Facilitate the coordination of communication efforts among various governmental departments (e.g. health, agriculture, and trade) and other stakeholders sharing responsibility for food safety at the national or other levels.

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RISK COMMUNICATION TYPES IN FOOD SAFETY CONTEXT

Type of incident:

**Emergency food safety incidents
(microbiological or physical contamination of foods)**

**Enduring food safety problems
(Mycotoxins)**

Response:

Rapid response

Sustained communication



MITIGATION OF RISKS

What we want to avoid:

1. Harmful behavior – two extremes (ignorance vs. panic);
2. Severe health, environmental, trade and economic implications;

How?

**Sticking with the principles of good risk communication:
Transparency, Openness, Responsiveness, Timeliness**

**Building the trust
and credibility**

**Inclusion
and dialog**

**Allow public
inspection**

**Messages adjusted
to the audience**

**Good
coordination**

**Adequate
planning**



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PLANNING CHECKLIST:

- ✓ Gather information and prioritize food safety issues;
- ✓ Identify your objectives/what you want to achieve;
- ✓ Identify and understand target audiences, and work with stakeholders;
- ✓ Develop the messages;
- ✓ Gather information about available people and resources for communication activities, and identify gaps in capacity and other resources;
- ✓ Identify communications channels and tools;
- ✓ Monitor and evaluate.

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COMMUNICATING ON MYCOTOXINS IN AFGHANISTAN'S ENVIRONMENT - CHALLENGES

- ✓ Highly sensitive political environment;
- ✓ Two key factors that influence panic, trust and tight regulations, are both questionable;
- ✓ Ability of population to self-educate themselves is limited and leaves too much room for rumors;
- ✓ Presence of mycotoxins influence Afghan economy and public health;

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COMMUNICATING ON MYCOTOXINS IN AFGHANISTAN'S ENVIRONMENT - OPPORTUNITIES

Opportunity for the Government to show:

- ✓ They care about its citizens;
- ✓ It is willing to take action to protect public health, as well as the economic interests and wellbeing of its citizens;
- ✓ For the first time Government is willing to tackle long-term problem, which nobody was dealing with before;
- ✓ Keeping people involved and informed is a trust-building process;
- ✓ Communicating successes - **WE ACHIEVED IT TOGETHER!**

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COMMUNICATING ON MYCOTOXINS IN AFGHANISTAN'S ENVIRONMENT – PHASING OF THE COMMUNICATION

Balance between urgency and risks;

Start with the audiences and messages that are the least risky and produce the biggest economic impact;

Start with the most risky communication when sure that communicators are educated enough;

Start with the most risky part when we can say that we already did something and when we can prove it;

Keep the focus on what we are doing about the problem and make sure we are doing enough!

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***Good communication
is a learning opportunity
and a trust building process!***

Andja Cosic

Thank you for your attention!

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Appendix XVI – Draft Risk Communications Proposal

DRAFT Proposal to Strengthen Risk Communications Capacity in Afghanistan (2 December 2016)

Background:

In December 2015, Afghanistan's Ministry of Agriculture, Irrigation and Livestock (MAIL), with support from the United States Agency for International Development (USAID), opened a laboratory for rapid assessment of Afghan agricultural products for mycotoxins, toxic byproducts of mold. The assessment found mycotoxins in Afghan agricultural products at levels that potentially impact human health and agricultural trade. USAID and K-State co-hosted a conference in New Delhi in 2016 to summarize results and identify next steps.

USAID is requesting support from K-State to develop a strategic communications plan and to build risk communication capacity for accurate, effective communications with Afghan citizens, government counterparts, and the international community on the presence of mycotoxins in Afghan agricultural products, the dangers posed, and the strategy developed by MAIL, Ministry of Commerce and Industry (MoCI) and Ministry of Health (MoH) to increase the safety of Afghan agricultural products for export and consumption.

Target audiences:

The target audiences for communications events and products produced include: (i) residents of Afghanistan, who might consume mycotoxins, (ii) Afghan farmers and agribusinesses, whose crops may be contaminated with mycotoxins, (iii) Afghan government employees at national, provincial, and district levels, who should be informed of and able to comment and disperse information on impacts of mycotoxins in Afghan agricultural products, and (iv) national government and private sector stakeholders in export markets for Afghan agricultural products.

Deliverables:

Kansas State University, working in conjunction with the USAID Office of Agriculture (OAG) and the Development Outreach and Communications Office (DOC), will: (i) arrange for a strategic communications team to collaborate intensively with a MAIL/MoCI/MoH ergot response team to create in-country capacity to address ergot contamination of agricultural products and related issues. A parallel research effort will develop and identify methods for repurposing and disposal of ergot-infected products. (ii) Build broader expertise in risk communications through internships for critical MAIL, MoCI and MoH staff with K-State communications experts over a longer term. With world class capabilities in plant pathology research, risk communications and extension, Kansas State University is well positioned to partner with USAID on this food security issue.

Methodology

(i) The Afghan ergot response team will work directly with K-State counterparts to create highly specific visual communications materials and methodology for distribution. In addition, risk communications training will be conducted, including tracking and response mechanisms to determine outcomes of rapid response situations. Once communications materials are created

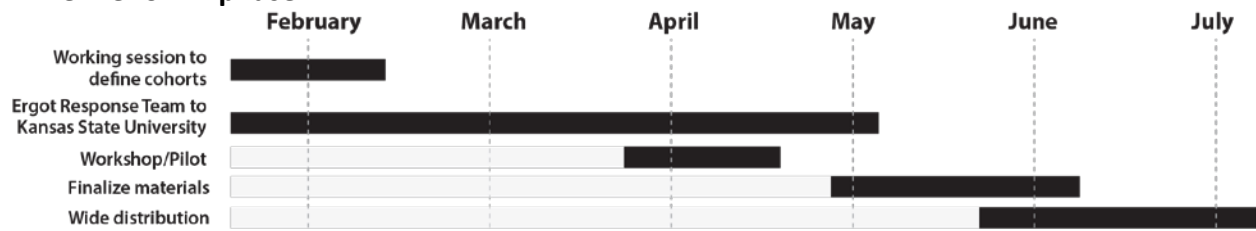
and validated, they will be made available on for use within the country through other USAID and Government sponsored programs. Elements of the first phase of the program include:

- Working session with USAID and ministry representatives in early 2016 to identify cohort members and define deliverables.
- Ergot response team members (PIOs and technical representatives from MAIL, MoCI and MoH) travel to K-State for 3-4 months. Develop materials for use in Afghanistan, including materials and messages for extension communications. Participate in training on risk communications and media tracking.
- Workshop in India in collaboration with USAID and participating ministries that includes targeted partners. Release pilot risk communications materials for validation and to confirm effectiveness.
- Train the trainer workshop coinciding with rollout of plans and training materials to support in-country communications.
- Use lessons learned from ergot response team experiences in Manhattan to develop programs for additional cohorts.

(ii) Develop an internship program of 4-6 month duration in Manhattan for critical MAIL, MoCI and MoH staff. Multiple (4-6) cohorts with members from each ministry will work with K-State mentors on the use of media tracking/monitoring tools (such as Nuvi and Meltwater) and responses to social and traditional media incidents. Cohort members will reside at the university, be selected based on USAID recommendations, and come from three general classes: administrators, public information officers, and technical/research specialists. The cohort approach facilitates relationship building and networking between ministries. Cohorts will not overlap during their time at K-State, with the entire set of 4-6 cohorts spending time over a 2-3 year period. Project focus may vary by cohort and will depend on current priority food safety and security issues within the country. Size of cohorts may vary (minimum of 3, 6 recommended). Associated with each cohort would be a Stakeholder’s meeting and a Train the Trainer workshop similar to those envisaged for the Ergot Response Team. Depending on the topic, members of a cohort may travel to other institutions in the United States for specialized experiences not available at K-State.

Training for the seven proposed cohorts would be complete within a four-year time frame. Each cohort would follow the general timeline below for the Ergot Response Team (1st phase). By training one cohort at a time each cohort could focus on a relevant current issue.

Timeline for 1st phase



Appendix XVII – 16 July 2016 Meeting – Discussion Summaries

XVII.1 Ministry of Agriculture, Irrigation and Livestock Discussion Summary

XVII.2 Ministry of Commerce and Industry Discussion Summary

XVII.3 Ministry of Public Health Discussion Summary



Post-conference Workshop on Food Quality and Safety
Risk Communication Session



Ministry of Agriculture, Irrigation and Livestock			
Questions	Answers	Level of Risk/Priority	Deadline
What do we know about Mycotoxins? What are the public health risks and economic risks?	Tested samples of raisins and pistachios showed contamination, up to 50% of samples. Almonds contaminated in 20% of samples. Contamination of wheat is up to 15% of samples. No contamination of milk (in the region covered by Kabul Dairy Union). Literature needs to be reviewed to help identifying the toxicity levels. Quantifying is still an issue - samples need to be homogenous. Which level of value chain have they been sampled? Monitoring of different levels of value chain, different locations and different actors and varieties needed. AflaSAFE/AflaGuard (6 months to 1 year lab), Biological control could be employed. Implementation of GAP should be the more immediate step training farmers and agribusinesses.	High, medium or low priority	(When we should start /how long preparation is needed?)
Whom to ask?/How to double-check?	Mycotoxin lab; Nuts and Raisins Export Union; PPQD within MAIL;		
What are our objectives?/What we want to achieve communicating?	Communicate within the MAIL about the problem. Identify the right person from ministries to follow up. Raise awareness of producers.		
Whom we are talking to?/Who are our audiences?	Internal audience, to be informed and skilled to introduce new practices and change procedures; Farmers; Food processing companies; Partners such as FAO; Scientists; Cooperatives and other farmers' associations;		
What are our messages?	Presence of mycotoxins influences farmers income. Levels of mycotoxins will decrease if pre-harvest and post-harvest practices change. Testing of grains and nuts is needed to protect health of Afghan citizens and to secure export.		
What are the challenges and risks we have to take into account?	MAIL capacity to communicate effectively. Panic has to be avoided. Messages well structured so there is no negative impact on domestic consumption.		



Post-conference Workshop on Food Quality and Safety
Risk Communication Session



Which communications tools and channels we are going to use?	Direct communication with farmers through extension workers. Formal trainings. Stakeholders meetings (i.e. exporters and producers); Brochures; Short educational videos;		
What needs to be done in your Ministry to make it capable to communicate about Mycotoxins?	Committee to be established; training needed; adequate planning; communication; inter-ministerial committee; funding.		
Who from your Ministry could take the lead in dealing with Mycotoxin issues, including communication?	Most likely PPQD, however it requires additional consultations prior final decision.		
How we will monitor our communication activities?	No answer at the moment;		
How we will evaluate the impact?	No answer at the moment;		

Ministry of Commerce and Industries			
Questions	Answers	Level of Risk/Priority	Deadline
What do we know about Mycotoxins? What are the public health risks and economic risks?	<ul style="list-style-type: none"> - Impacts exports and reduces incomes; - Diminishes country image; - Public health risks; - Undermine counter narcotics efforts. 	Exports - medium, imports - high priority.	(When we can start /how long preparation is needed?)
Whom to ask?/How to double-check?	- MAIL, MoPH, MoCI		
What are our objectives?/What we want to achieve communicating?	<ul style="list-style-type: none"> - Education and training of exporters; - HACCP; - Responsiveness (traceability); - Guarantee of safe food, consumer protection; - Compliance setting. 		
Whom we are talking to?/Who are our audiences?	Exporters; Processors; Producers; <u>Value Chain</u> actors; Importers; Distributors, Wholesalers; Civil society;		
What are our messages?	Exporters: Adherence to standards will allow for the expansion to new markets. Importers: Consumer safety; Social Responsibility; Business longevity.		
What are the challenges and risks we have to take into account?	<ul style="list-style-type: none"> - Over or underestimate audience capacity. - Correct understanding of business practices; - Decrease in trade volumes. 		
What needs to be done in your Ministry to make it capable to communicate about Mycotoxins?	<ul style="list-style-type: none"> - EPAA needs to be strengthened; - Strengthen technical unit at MoCI that has laboratory facilities and often clears products for export (also referred as Association of exports of dried fruits); - Identify international best practices; 		
Who from your Ministry could take the lead in dealing with Mycotoxin issues, including communication?	To be identified (technical unit alluded to above).		
Which communications tools and channels we are going to use?	Printed instructions and info brochures about standards, regulations, best practices etc.; Trade groups; Civil society;		



Post-conference Workshop on Food Quality and Safety
Risk Communication Session



	Development programs.		
How we will monitor our communication activities?	Monitoring number of rejected products intended for export. New markets accessed.		
How we will evaluate the impact?	Level of mycotoxin related diseases decreased. Increased export sales.		

Ministry of Public Health			
Questions	Answers	Level of Risk/Priority	Deadline
What do we know about Mycotoxins? What are the public health risks and economic risks?	<ul style="list-style-type: none"> - Health risks: liver cancer, kidney failure, immunity suppression, children stunting. - Economic risks: increased costs of medical treatments. 	Low priority	Further assessment and preparations needed
Whom to ask?/How to double-check?	<ul style="list-style-type: none"> - Internally: nutrition department, health promotion, food safety. - External partners: MAIL, FAO, WHO, and other relevant partners including private sector. 		
What are our objectives?/What we want to achieve communicating?	<ul style="list-style-type: none"> - Minimize the risk. - Improve health and nutrition status of the people. 		
Whom we are talking to?/Who are our audiences?	<ul style="list-style-type: none"> - Health providers/medical workers. - General population. - Decision makers. 		
What are our messages?	<ul style="list-style-type: none"> - What is mycotoxin? - How does it contaminate food? - What are its health implications? - How it can be prevented? 		
What are the challenges and risks we have to take into account?	<ul style="list-style-type: none"> - Lack of baseline information. - Lack of coordination. - Competing priorities (other health issues that are considered more urgent). - Lack of human capacity. - Lack of funding. 		
Which communications tools and channels we are going to use?	Depending on the type of audience: <ul style="list-style-type: none"> - mass media, - brochures, - trainings, - public gatherings, mosques. 		
What needs to be done in your Ministry to make it capable to communicate about Mycotoxins?	<ul style="list-style-type: none"> - Generate data/evidence. - Develop capacity to communicate. - Allocate resources. - Coordinate with line departments and ministries. 		
Who from your Ministry could take the lead in dealing with Mycotoxin	<ul style="list-style-type: none"> - Food safety, - Nutrition, and - Health Promotion Departments. 		



Post-conference Workshop on Food Quality and Safety
Risk Communication Session



issues, including communication?			
How we will monitor our communication activities?	Health Promotion and Public Relations Departments.		
How we will evaluate the impact?	- Through measuring the burden of associated diseases. - Level of public awareness.		