





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 μ g/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 μ g/kg) and two pistachio (2.5 μ g/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 were 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 highquality 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.

	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 re- gion	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	Samples Analyzed
		of Samples	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 ker-	21	21
	nels)		
A07	Other almonds	14	12
	TOTAL	96	94

Table 3. Raisin samples.

	Type of Sample	Total Number of Samples	Samples Ana- lyzed 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 Ana- lyzed in Austria
small seeds, often exported to the former Soviet Union to make cognac (Kvass)-like product]	<u> </u>	•
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
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
Small red raisin or currant (sun dried and stirred in dirt, locally used raisins in rice dishes and baked goods)	18	18
Other or mixed raisins	4	4 201
	small seeds, often exported to the former Soviet Union to make cognac (Kvass)-like product] 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] 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) Small red raisin or currant (sun dried and stirred an dirt, locally used raisins in rice dishes and paked goods)	SumplesSamplesSmall seeds, often exported to the former Soviet Union to make cognac (Kvass)-like product]Image: 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]19Sun-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)14Small red raisin or currant (sun dried and stirred in dirt, locally used raisins in rice dishes and paked goods)18Other or mixed raisins4

 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 ana- lyzed 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

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

	Aflatoxin				Ochratoxin			
Analysis	# of	<eu<sup>b</eu<sup>	>EU,	>US ^d	# of	<lod<sup>e</lod<sup>	<eu< td=""><td>$> EU^{f}$</td></eu<>	$> EU^{f}$
	samples ^a		<us<sup>c</us<sup>		samples			
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 ¹	-	-	-	-	219	26	73	1

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

^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.

¹Assay conducted at Kansas State University with a Viacam ELISA test kit.

a level above 8.0 μ g/kg (ppb) – the EU maximum allowable contamination. Fumonisin B₂ also was detected in ten samples at levels ranging from 5.4-25.7 μ g/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 μ g/kg of aflatoxins, with one sample contaminated at nearly 4,000 μ g/kg. Two additional samples were contaminated at levels less than the 4 μ g/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 μ g/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 (< $0.8 \mu g/kg$) 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 guide-line of 3 μ g/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 μ g/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 produc-
ing genera.

Commodity	Fusarium	Alternaria	Aspergillus	Penicillium	Ergot	
Wheat	Beauvericin	Tenuazonic acid	3-Nitropropionic acid	Mycophenolic acid	Ergocristine	
	Enniatin A	Alternariol	Kojic acid	Agroclavine	Ergocristi- nine	
	Enniatin A ₁	Alternariol methyl ether	Sterigmatocystin	Chanoclavin	Ergometrine	
	Enniatin B	Tentoxin	Methoxy-sterigmato- cystin	Elymoclavine	Ergome- trinine	
	Enniatin B1	Altersetin	Averantin	Citrinin	Ergosin	
	Epiequisetin	Altersolanol	Averufin	Secalonic acid D	Ergosinin	
	Equisetin	Altertoxin I	Norsolorinic acid	Questiomycin A	Ergotamine	
	Chrysogin	Macrosporin		Quinolactacin A	Ergotami- nine	
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		
			Malformin C	Andrastin B		
Raisins		Tenuazonic acid	Malformin A	Mycophenolic acid		
		Alternariol	Malformin A ₂	Mycophenolic acid IV		
		Alternariol- methylether	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

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 upfront 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 longterm 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 develop that enable rapid, transparent tests of targets 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 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 Asiab 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)

	Producer	Trader	Processor	Distributor	Exporter	Importer	Government	Health/Vet	Consumer
Technical Experts							~	~	
Highly Literate	~	~	~	~	~	~	~	✓	~
Average Literate	~	~	~	~	✓	~	~	~	~
Nominally Literate	~	~	~						~
Illiterate	~								~
Training							×	X	
Technical Info	×	×	×	×	×	×	×	X	×
Public Education	×								×
Advocacy		×	×	×	X	×	×	X	
Crisis Commun	×	×	×	×	×	×	×	×	×
Health Risk									
Financial Risk									
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

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

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 B_1 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."*

<u>Incidence of liver and kidney cancer in Afghanistan</u>. According to World Health ranking (<u>http://www.worldlifeexpectancy.com/cause-of-death/liver-disease/by-country/</u>), Afghanistan ranks 6^{th} in the world for liver cancer and 3^{rd} 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. <u>http://www.elisa-tek.com/diagnostic-testing-kits/mycotoxins/</u>) 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 GIRoA'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.

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

VI. ILLUSTRATIVE DURATION, TIMING AND SCHEDULE

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

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

MODIFICATION OF ASSISTANCE		Page 1 of 3	
1. MODIFICATION NUMBER	2. EFFECTIVE DATE OF MODIFICATION	3. AWARD NUMBER:	4. EFFECTIVE DATE OF AWARD :
02	See block 15	AID-0AA-L-14-00002	01/01/2014
5. GRANTEE:		6. ADMINIST	ERED BY:
2 Fairchild H	earch and Sponsored Progr	ams Offic M/O 300	usition and Assistance e of Acquisition and Assistance AA/BFS, Room 512-C, SA-44 Pennsylvania Ave, N.W. hington, D.C. 20523
DUNS NO 929773554 TIN NO. : 48077175	1 LOC NO. : 23A6P		
7. FISCAL DATA: Budget Fiscal Year: Operating Unit: Strategic Objective Team/Division: Benefiting Geo Are Object Class:		GLAAS Requ 9. PAYMENT U.S. Agen Office of I SA-44,M/ 1300 Pen	L OFFICE: BFS/ARP; aisition: REQM-BFS-15-000071 OFFICE: cy for International Development Financial Management CFO/CMP nsylvania Avenue, NW on DC 20523
10. FUNDING SUMMA	RY:	Obligated A	mount Total Est. Amt.
Amount Prior to the	is Modification:	\$1.950.00	0.00 \$5.000.000.00
Change Made by t	his Modification:	\$ 1.220.53	5.00 \$ 0.00
New/Current Total:		\$3,170,53	5.00 \$5,000,000.00

11. DESCRIPTION OF MODIFICATION

1) The purpose of this modification is to provide incremental funding in the amount of \$1,220,535.00 to support the program for Innovation Lab for Reduction of Post-Harvest Loss. which will be received via Mission field support funding (USAID/Afghanistan) (buy-in) to support the program for Innovation Lab for Reduction of Post-Harvest Lost. The total obligated amount increased from \$1,950,000.00 to \$3,170,535.00.

2) Background

The purpose of this activity to assist USAID/Afghanistan and the Government of the

12. THIS MODIFICATION IS ENTERED INTO PURSUANT TO THE AUTHORITY OF AS AMENDED. EXCEPT AS SPECIFICALLY HEREIN AMENDED, ALL TERMS / REFERENCED IN BLOCK #3 ABOVE, AS IT MAY HAVE HERETOFORE BEEN A FORCE AND EFFECT.	
13. GRANTEE X IS IS NOT REQUIRED TO SIGN THIS DOCUM EFFECTED HEREIN	ENT TO RECONFIRM ITS AGREEMENT WITH THE CHANGES
14. GRANTEE:	15. THE UNITED STATES OF AMERICA U.S. AGENCY FOR INTERNATIONAL DEVELOPMENT
ву:	ВҮ:
	Charles Jackson
(Name Typed or Printed)	(Name Typed or Printed)
TITLE:	TITLE: <u>Agreement Officer</u>
DATE:	DATE:

MODIFICATION OF ASSISTANCE	-
CONTINUATION PAGE	

ASSISTANCENO. AID-OAA-L-14-00002	MODIFICATION NO.	
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 alfatoxin (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 alfatoxin 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.

PAGE NO. 02 of 03 AID-OAA-L-14-00002 Modification #02

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 <u>https://www.youtube.com/watch?v=J7kut5N3ubw</u>

Risk Assessment Related to Pathogenic Hazards in Food Processing https://www.youtube.com/watch?v=2ITh2PAsalE

خام مقانير ط PH Meter (You will be using this) https://www.youtube.com/watch?v=BVbKcQTZIKs

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

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 <u>https://www.youtube.com/watch?v=Zy1kt6EKOWI</u>

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=V11BtOOrxRY

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

Concentrations Part 3 https://www.youtube.com/watch?v=yFn59OMUgOU

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=1BdVmIATI2w

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=nNjlBCnpGZ4

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=zl4khlJhCd8

ELISA Tutorial 5: Preparing ELISA Data in Excel for Analysis with GraphPad Prism https://www.youtube.com/watch?v=l9t0812CeRg

ELISA Tutorial 6: How to Analyze ELISA Data with GraphPad Prism https://www.youtube.com/watch?v=5IqqpKSnXfl

Mycotoxin MycoSep Columns (You will be using this) https://www.youtube.com/watch?v=3QBkCLZIvDU

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=pzUi6gxuy3g

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



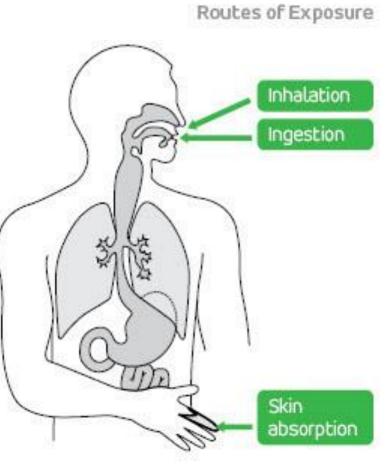
General Information

- 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



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

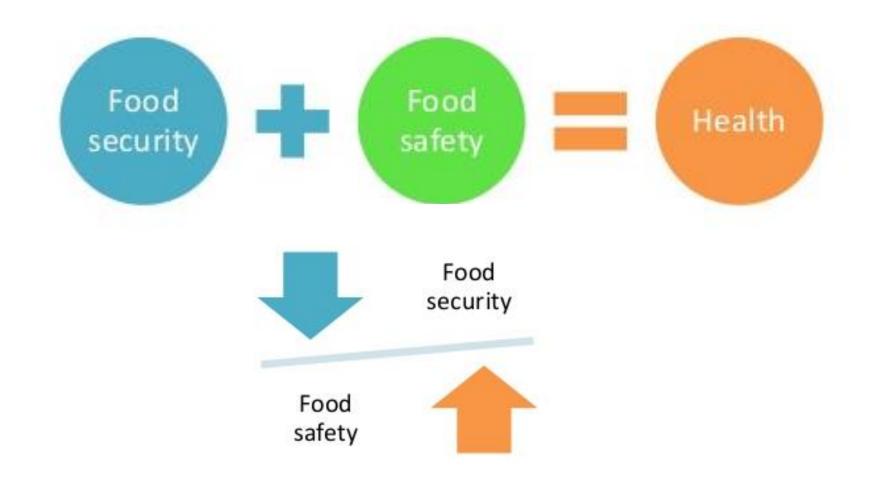
indirect exposure

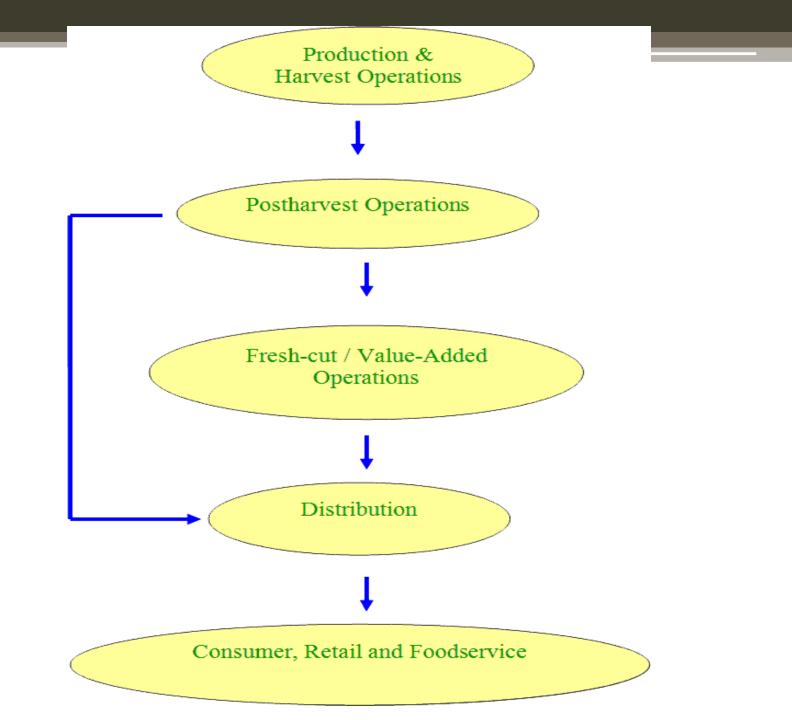
exposure

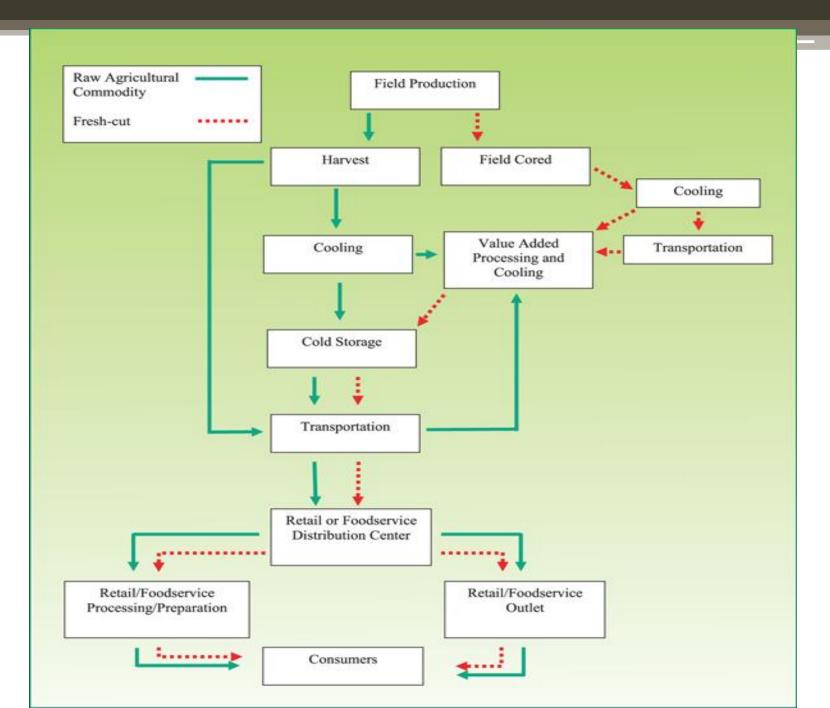
- Food Ingredients
- Residues in animal products milk, eggs, edible organ tissues



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

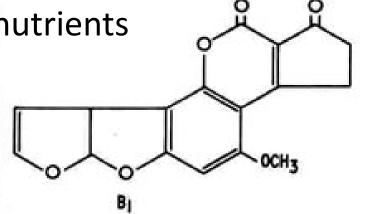




Aflatoxins

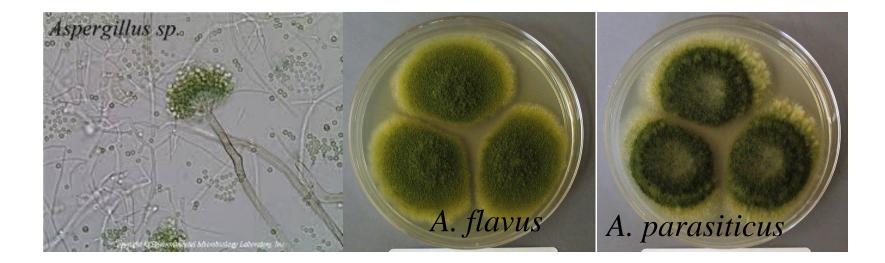
Aflatoxins are heptocarcinogens

- 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



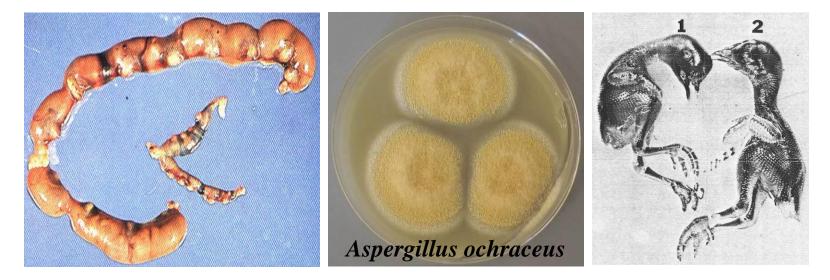
Aflatoxins

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



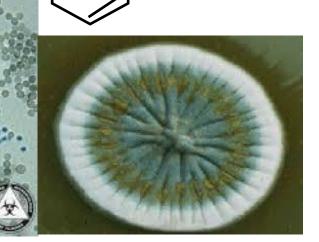
Ochratoxin A

- 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, COOH O | | || raisins and wheat



 $\mathcal{J}H_{2}$

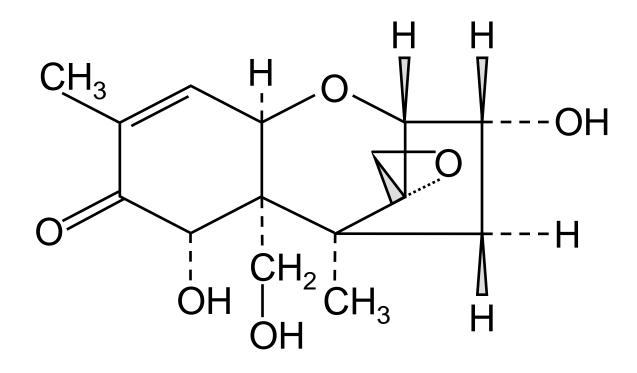
Deoxynivalenol/Vomitoxin

- 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

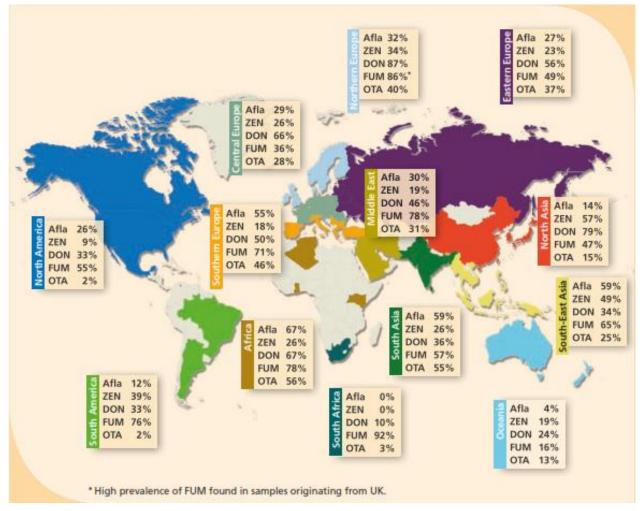
<u>Mycotoxins</u>	<u>Molds</u>
Aflatoxins	Aspergillus flavus, A. parasiticus, A. nomius
Ochratoxin	Aspergillus ochraceus, A. niger Penicillium verrucosum
Fumonisins	Fusarium verticillioides (moniliforme) F. proliferatum, F. subglutinans, F. tricinctum
Deoxynivalenol (DON, Vomitoxin)	Fusarium graminearum, F. culmorum F. pseudograminearum

Geographic Pattern of Mycotoxin Occurrence



www.biomin.net

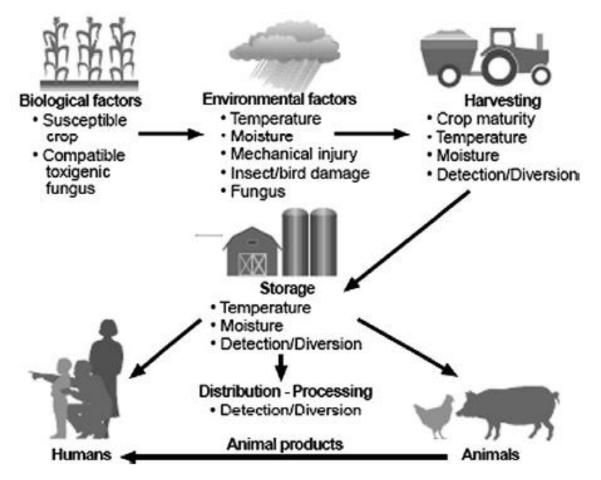
Mycotoxin Global Occurrence in 2013



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

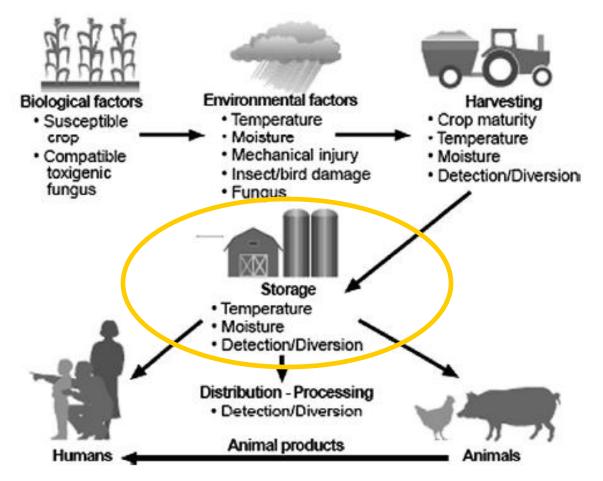
www.biomin.net

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
 - Fumonisins





نظارت و ارزیابی گدام غله جات

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

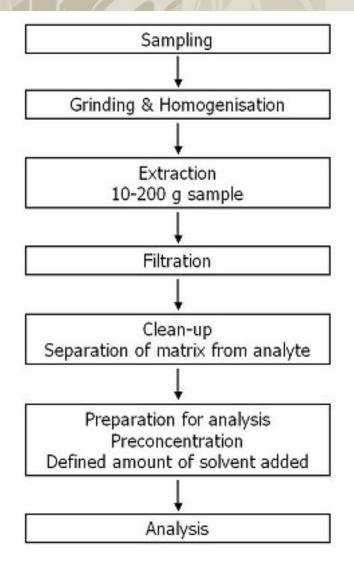
STACKING OF BAGS IN WARE-HOUSE



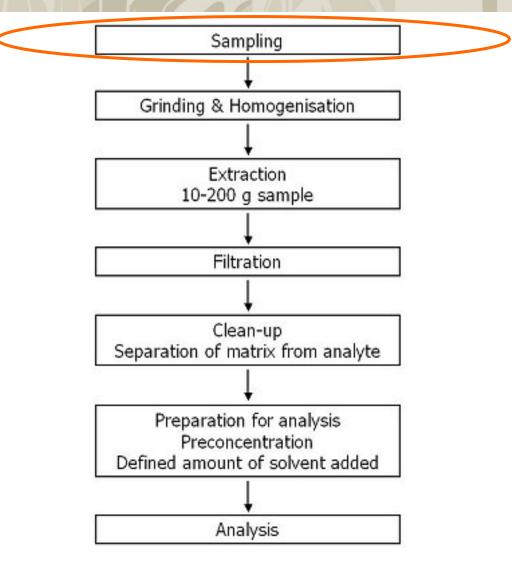
Correct Method

Incorrect Method

Mycotoxin Analysis - Main Steps



Mycotoxin Analysis - Main Steps

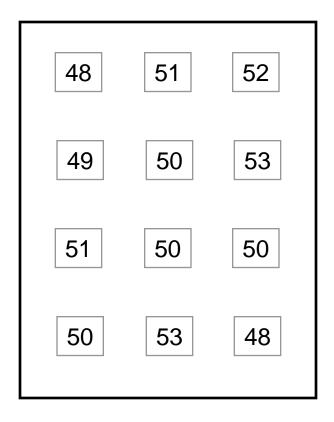


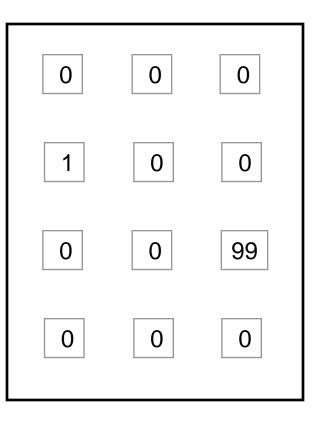
Sampling

- 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



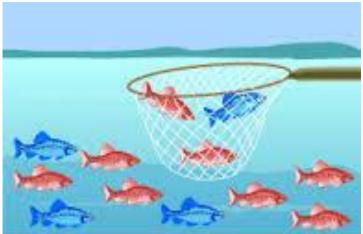


Protein

Aflatoxin

Sampling - Representative Sample

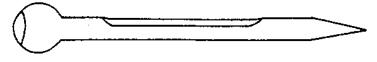
- In order for a sample to be representative it must:
 - Be obtained with equipment and procedures designed to obtain sample from all areas of the lot
 - Be of appropriate size
 - Be adequately identified
 - Be handled in such a way as to maintain its representativeness



Sampling - Representative Sample

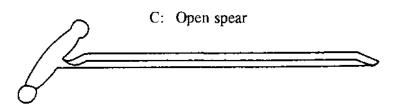
Probes and bag triers

A: Closed spear for sampling large grains such as maize



B: Closed spear for sampling small grains such as wheat





D: Double-tube spear





Sampling - Representative Sample

Sampling procedures







Sampling Devices

Probes

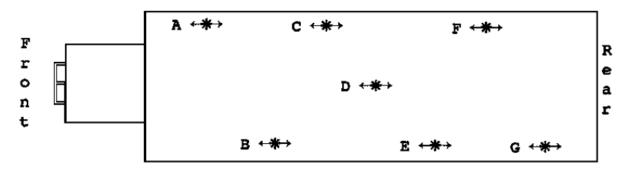
- Standard lengths 5, 6, 8, 10, and 12 feet
- The depth of the carrier defines the length of the probe used

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

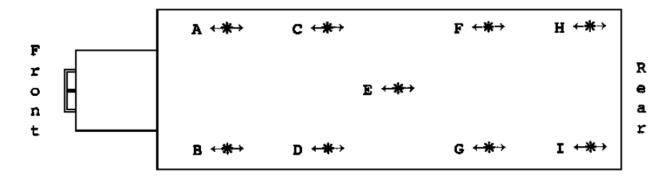
Other Containers - Use grain probes that will reach the bottom of the container.

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



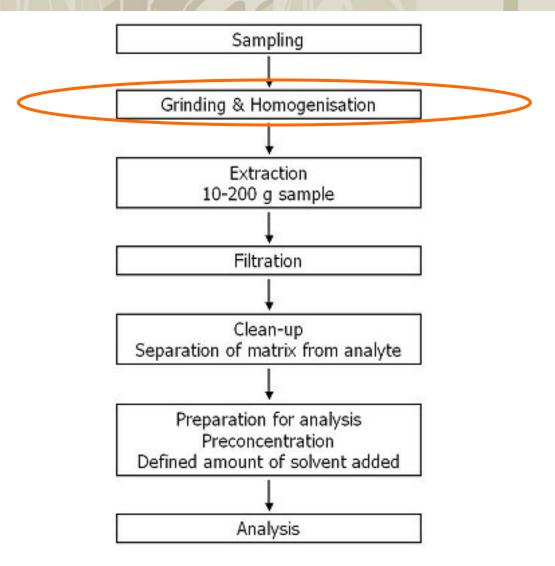
Sampling - Sample Size

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	

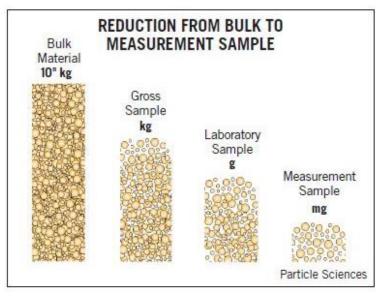
Truck Load = About 900 bushels of corn Each corn bushel = 56 lbs 908 g sample = 0.04% of the load! But never less than 1 kg!!

Mycotoxin Analysis - Main Steps

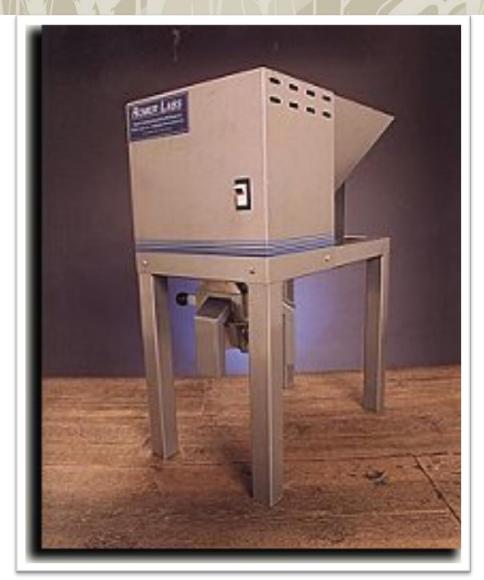


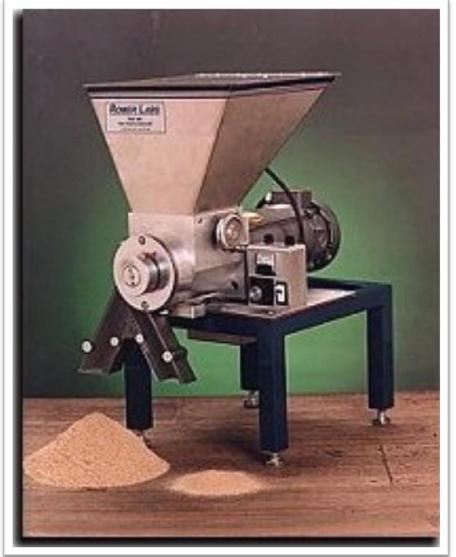
Sample Preparation - GIPSA

- 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

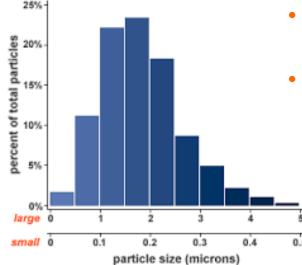




Size Reduction in the Field

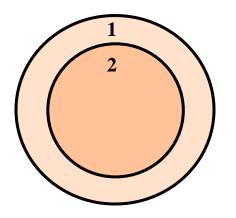






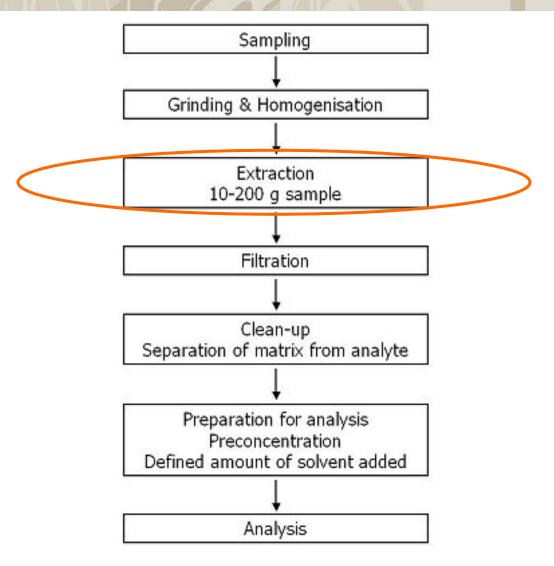
- The samples should be ground in its totality (300 g)
- According to Romer Labs:
 - Sample for analysis: 75% should pass through a 20-mesh screen (850 μm)
 - 5 min in the food processor: 52.56% of samples was smaller than 850μm
 - 3 min in the ostar grinder: 81.22% of samples was smaller than 850μm

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

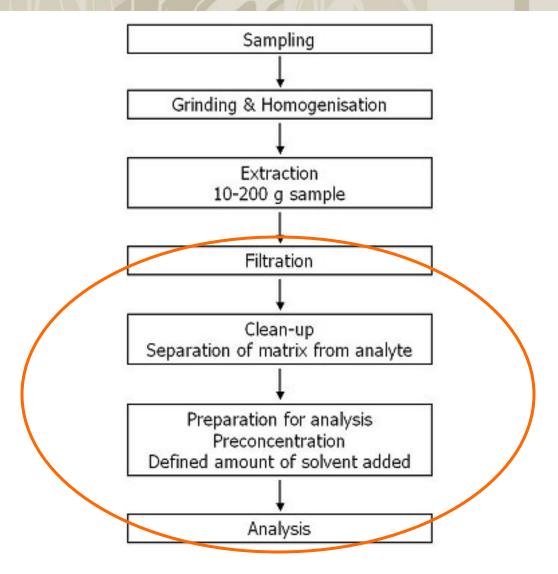


Mycotoxin Extraction

- Extraction solvent
 - Aqueous phase (phosphate buffer)
 - Organic solvent
 - Combination of both
 Methanol and Water Aflatoxins, Ochratoxin, Fumonisins
 - 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	Number of	Aflatoxin Levels	Fumonisin Levels
Samples	samples	(Average; Range)	(Average; Range)
At harvesting	8	5.56ppb;	0.15ppm;
		<2ppb - 21.4ppb	All samples <0.3ppm
After screening	4	3.65ppb;	0.15ppm;
		<2ppb - 6.34ppb	All samples <0.3ppm
At beginning of	9	4.31ppb;	1.25ppm;
storage (day 0)		<2ppb - 8.86ppb	<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 Andreia Bianchini, PhD University of Nebraska - Lincoln and Debra Frey, MSc Kansas State University









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

27 JULY, 2015



Lincoln

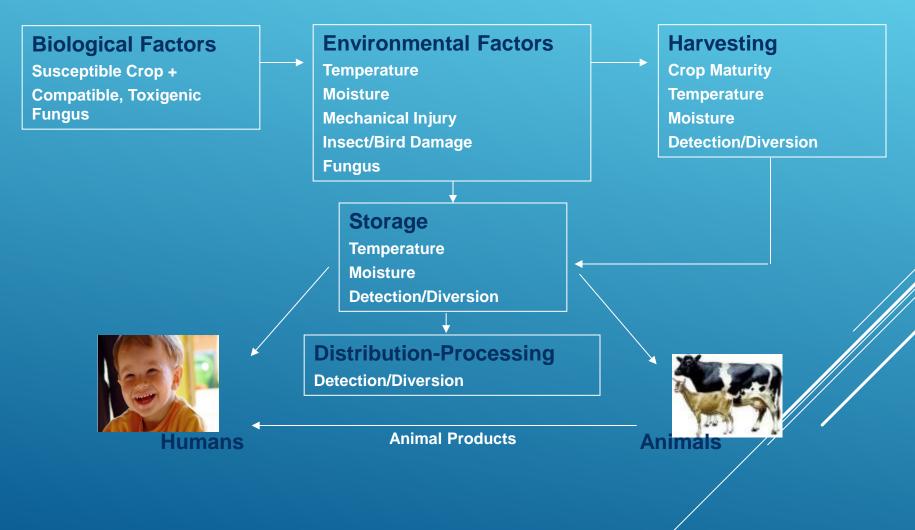
PURPOSE

- 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 <u>mycotoxins</u> that are produced by several Aspergillus species of fungi, the major ones are <u>Aspergillus flavus</u> and <u>Aspergillus parasiticus</u>.
- 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



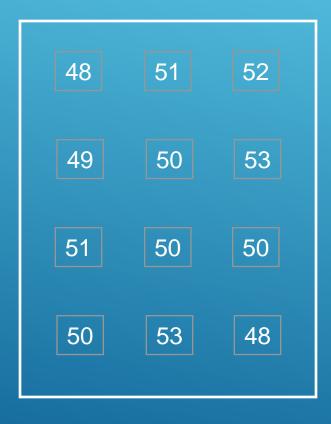


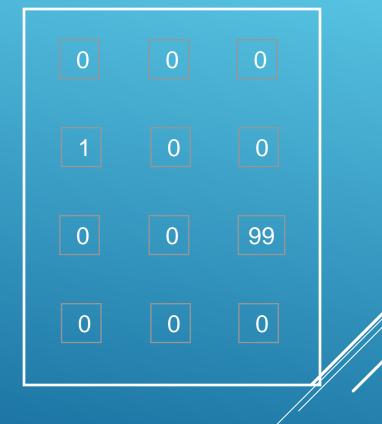
SAMPLING

- 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





Protein

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.















PHL Innovation Lab Afghanistan

Sampling Procedure Protocol

27 July 2015















OULTINE

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 pilled 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 gridding and at the end of the process).



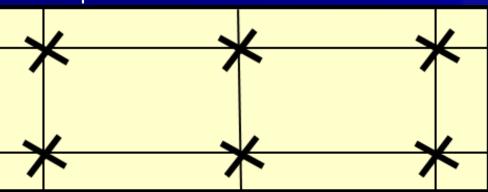




Sampling points

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 pilled, 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.

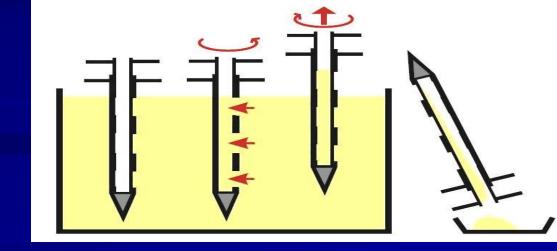
Grid Pattern



Using probe sampler:

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

- a. Insert the sampler into the product bag/container (A)
- b. Rotate the inner tube through 180° (B), to open the sampler.
 The product can now flow into the slot sampler.
- c. Rotate the inner tube through 180° to close the sampler and withdraw the sampler (C).
- d. 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)

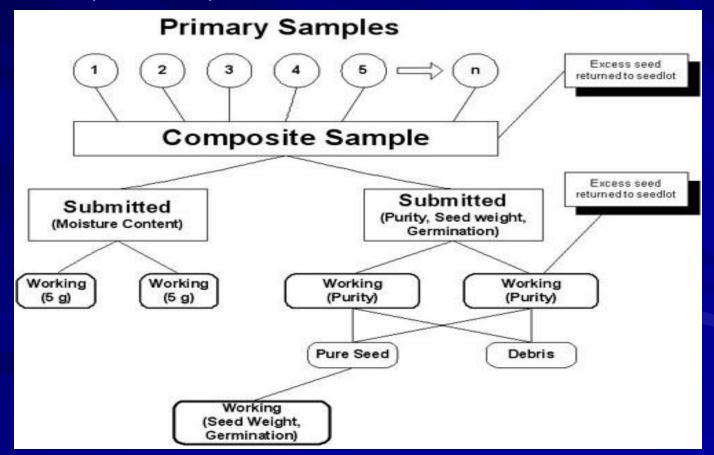
In order to obtain a representative sample from a lot a sufficient number of sub-samples must be taken using the following rule:

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.

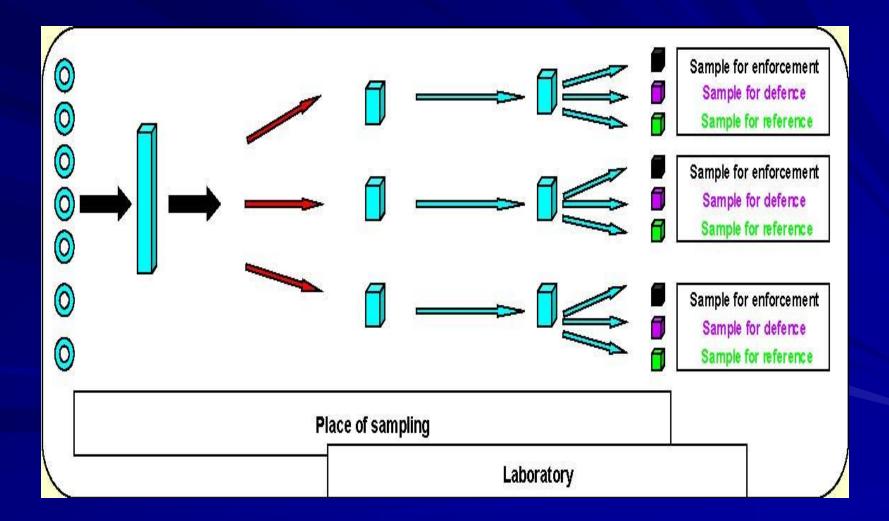


Take Composite Sample

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 subsamples 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

SUSAID KANSAS STATE Nebičiška	
Feed the Future Innovation Lab for the	
Reduction of Post-Harvest Loss	
Individual sample information	
Sample description: What Nata Rabins	
Date of sample collection: 3 30 15 MN D0 YY	
Days sample has been stored 75 (ince problem of hower, Timore)	
Type of storage. Begin	
Sample origin	
Location: Kaba/ (ef semple provider)	
Produced Bought X Other	
For "Other" plasse specify	

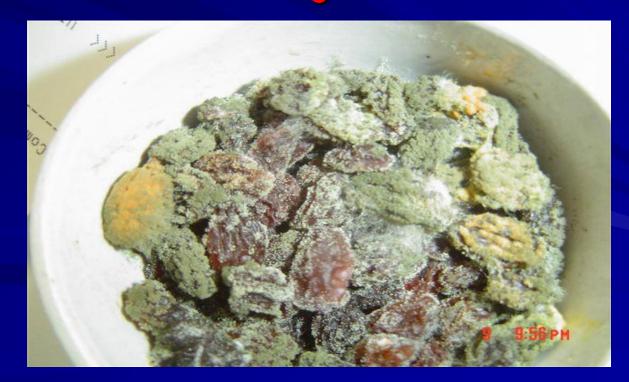
Place the 500 g sample in sterile plastics bags, properly labeled

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	6
	Herat, as recommended by grain traders or farmers.	
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	17 - 28
	Raisin	
R03	High Quality Shundurkhani Raisin	17 - 29
	(Golden-High Value)	
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-	15 - 25
	Sharif)	
A02	Shokorbai Hard-shell Almonds	15 - 25
A03	Abdul Wahidi Almonds (Mazar-i-Sharif)	15 - 25
A04	Qambari Amonds	15 - 25
A05	Ghorbandi Alomonds	15 - 25
A06	Sangaki and Murawaji Almonds (smaller	15 - 25
	kernels)	
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.

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 Web:
 http://www.romerlabs.com



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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.





Pipette 200 μL conjugate solution into dilution wells



Add 100 μL of each standard or sample extract into the dilution wells.

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

Wash 5 times with distilled/deionized water

Tap dry washed wells



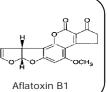
Pipette 100 µL substrate solution into the antibody coated wells, incubate at RT for 5 minutes



Pipette 100 μL stop solution into the antibody coated wells

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

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/			`		
	Perform	formance Characteristics:			
	LOD:	3 ppb for corn and other commodities			
		5 ppb for Sorghum			
		6 ppb for DDGS			
	LOQ:	4 ppb			
	Range:	4-40 ppb			
			Γ		

Sample Preparation / Extraction

- 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.
- 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.
- 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.
- 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 μ L/well or 2 mL/strip) and place in a separate container (e.g. reagent boat when using the 8channel pipettor). Using an 8-channel pipette, dispense 200 μ L of Conjugate into each blue/green-bordered Dilution Well.
- 4. Using a single channel pipettor, add 100 μ L of each standard or sample into the appropriate Dilution Well containing 200 μ L of Conjugate. Use a fresh pipette tip for

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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 bluecapped bottle (~120 μ L/well or 1 mL/strip) and dispense into a separate container (e.g. reagent boat for an 8channel pipettor). Pipette 100 μ L 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 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 doseresponse 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

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- *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 100μL and 200μL 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 8channel 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 enzymelinked 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,

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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: B_1 , B_2 , G_1 and G_2 , which are named for their respective innate fluorescent properties. Aflatoxin B_1 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 B_1 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 enzymelinked 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 antibodycoated 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

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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.
- 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.
- 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.
- 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

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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 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.®

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AgraQuant[®] Ochratoxin Assay 2/40

Order No.: COKAQ2000/COKAQ2048

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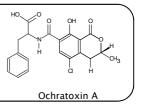


AgraQuant[®] Ochratoxin Assay 2/40 Competitive ELISA



Order #: COKAQ2000/COKAQ2048

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



Short Instruction:



Pipette 200 μL conjugate solution into dilution wells

Add 100 µL of each standard or sample extract into the dilution wells.

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

Wash 5 times with distilled or deionized water

Tap dry washed wells

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

Add 100 µL stop solution into the antibody coated wells

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

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

Sample Preparation / Extraction

- 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.
- 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.
- 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 μ L/well or 2 mL/strip) and place in a separate container (e.g. reagent boat when using the 8channel pipettor). Using an 8-channel pipette, dispense 200 μ L of Conjugate into each blue/green-bordered Dilution Well.
- 4. Using a single channel pipettor, add 100 μ L of each standard or sample into the appropriate Dilution Well containing 200 μ L 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

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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 bluecapped bottle (~120 μ L/well or 1 mL/strip) and dispense into a separate container (e.g. reagent boat for an 8channel pipettor). Pipette 100 μ L 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.
- 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.

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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 Oppb 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

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- *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 100μL and 200μL 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 8channel 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 enzymelinked 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.

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Ochratoxin

Ochratoxin, produced mainly by the fungi Aspergillus ochraceous 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 enzymeconjugated 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.

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- 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.
- 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:

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Romer Labs Singapore Pte. Ltd.	Fax: (65) 6275 5584	
3791 Jalan Bukit Merah #08-08	Web: http://www.romerlabs.com Email: salesasia@romerlabs.com	
e-Centre@redhill,		
Singapore, 159471		

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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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[®] T-2/HT-2 Toxin Assay 25/500

Order No.: COKAQ6100

Romer Labs Singapore Pte. Ltd.

- Tel: (65) 6631 8018
- Fax: (65) 6275 5584
- Web: http://www.romerlabs.com



For further information please contact:

Technical Services Romer Labs Singapore Pte. Ltd. 3791 Jalan Bukit Merah #08-08 e-Centre@redhill, Singapore, 159471 Tel: (65) 6631 8018 Fax: (65) 6275 5584 Web: http://www.romerlabs.com Email: salesasia@romerlabs.com

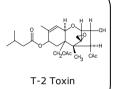


AgraQuant[®] T-2/HT-2 Toxin Assay 25/500 Competitive ELISA

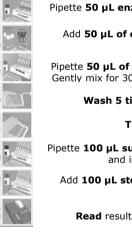


Order #: COKAQ6100

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 soyabeans as well as in some cereal-based products.



Short Instruction:



Pipette 50 µL enzyme conjugate into the microwells

Add **50 µL of each diluted standard or sample** into the microwells

Pipette **50** μ L 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 $100 \; \mu L \; stop \; solution$ into the antibody coated wells

Read results at 450 nm with an ELISA reader

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Performance Characteristics: I OD:

29 ppb (corn)

57 ppb (oats)

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

- 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.
- Add 100 mL of 70 % methanol and seal jar. Note: Samples 3. should be extracted in a ratio of 1:5 (w:v) of sample to extraction solution respectively.
- Vigorously shake the jar for 3 minutes. 4.
- Allow sample to settle, then filter the top layer of extract 5 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.
- The sample is ready for testing without further preparation. 7.





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.

- 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.
- 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.
- 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.





- 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.
- Measure the required amount of Substrate from the Substrate bottle (~120 µL/well or 1mL/strip) and dispense into a separate container (e.g. reagent boat for an 8channel 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 (~120 μ L/well or 1 mL/strip) and dispense into a separate container (e.g. reagent boat for an 8-channel pipettor). Pipette **100** μ L of Stop Solution into each microwell strip using an 8-channel pipettor. The color should change from blue to yellow.
- 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)





- 500 ppb (based on calibrators'
ncentration) - 500ppb (corn)
– 500ppb (oats)
01 7

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

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- 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 µL and 200 µL 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 8channel 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 soyabeans 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

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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 AgraOuant[®] 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 vellow. 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.

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Precautions

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

For further information please contact:

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

Tel: (65) 6631 8018 Fax: (65) 6275 5584 Web: http://www.romerlabs.com Email: salesasia@romerlabs.com

Warranty

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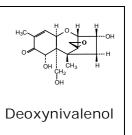
AgraStrip[®] Deoxynivalenol (DON) Quantitative Test



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

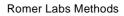
Deoxynivalenol (DON) is a type B trichothecene. DON is produced by fungi of the Fusarium genus, particularly Fusarium graminearum. This mycotoxin occurs predominantly in grains such as wheat, barley, oats, rye, and maize. DON is highly toxic, levels above 1 ppm are considered potentially harmful to swine. 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 vomition 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.





- 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):

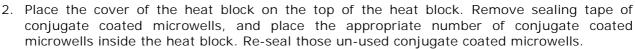
- 1. Weigh out 10g of ground sample into a Whirl-Pak[®] bag.
- 2. Add 40mL of distilled or de-ionized water and close Whirl-Pak[®] bag. Note: Samples should be extracted in a ratio of 1:4 (w:v) of sample to extraction solution respectively.
- 3. Vigorously shake for 1 minute.
- 4. Allow sample to settle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



- 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

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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 100μ L 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

For further information please contact:	
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Warranty

The user assumes all risk in using Romer Labs[®] products and services. Romer Labs[®] will warrant that its products and services meet all quality control standards set by Romer Labs[®], and Romer Labs[®] 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® shall be in no way responsible for the proper use of its products. Romer Labs® 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[®].

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









PHL Innovation Lab		
Afghanistan		
TITLE: General Laboratory Safety Precautions Concerning Mycotoxin Analysis		
Written by: Luis Sabillón	Edited by: Andréia Bianchini	
Effective date: 05/11/2015	Version: <u>1</u>	

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: Luis Sabillon	Edited by: Andréia Bianchini	
Effective date: 05/11/2015	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 pilled 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 subsample 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 gridding 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 pilled, 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.

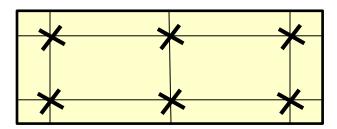


Figure 1. Grid Pattern

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

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

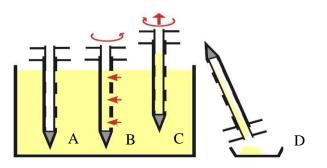


Figure 2. Using a sampler

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.

CUSAID KANNAN STATE Nebraska	
Feed the Future Innovation Lab for the Reduction of Post-Harvest Loss	
Individual sample information	
Sample description The Market	
Date of sample collection 3 30 16 M4 00 VV	
Days sample has been stored 75 (inceptoteen charve, Finner)	
Type of storage.	
Sample orgin	
Location Kisled	
Produced Bought X Other	23
For "Other"	2.9

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.



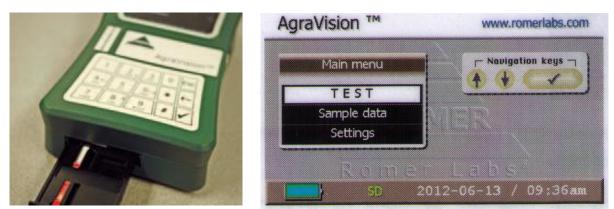




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Afghanistan		
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Edited by: Andréia Bianchini		
Version: <u>1</u>		

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.

1.	- analyte not assign	
2.	– analyte not assign	ied -
- Coloct 1	tem with [2]/[8] or page with [4]/ ith [/], clear assignment with [4]	TEG], assign sample/

- 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 meu 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.

Sample-ID: scan	next	more ABC
08/15-4711	Mustermann, Ma	К
edit with CO91.	delete with [+], switch	input mode with [*], go

- 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.



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









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

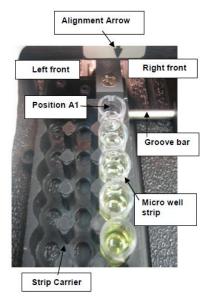
Run Test	Manage Tests
🐮 Settings	X Utilities

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		Ca	ncel
3 - TSH	, ,		
4 – TFSH/FSH VAST		^	<<
5 – T3-Uptake			
6 – LH/LH VAST		V	>>
7 - PRL	L		
8 - hCG		Se	elect
9 - Digoxin			
		B	y #

- 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 <u>all the way to left</u> 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 I	nnovation 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/COKAQ	21048)		
Written by: Luis Sabillón	Edited by: Andréia Bianchini		
Effective date: 05/11/2015	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.

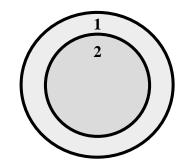


Figure 1. Procedure for reducing sample size.

- 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.

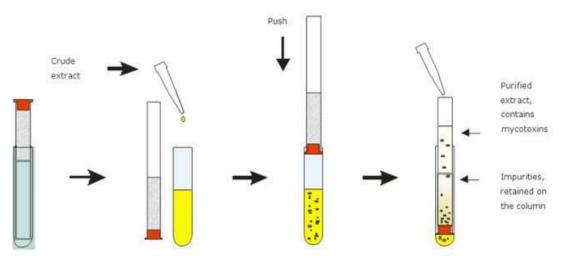


Figure 2. Procedure for using the MycoSep 112 columns

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 μ L/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 μ L of Conjugate into each blue/green-bordered Dilution Well.
- 4. Using a single channel pipettor, add 100 μ L of each standard or sample into the appropriate Dilution Well containing 200 μ L 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 μ L/well or 1 mL/strip) and dispense into a separate container (e.g. reagent boat for an 8- channel pipettor). Pipette 100 μ L 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 μ L/well or 1 mL/strip) and dispense into a separate container (e.g. reagent boat for an 8-channel pipettor). Pipette 100 μ L of 5 top 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 100μ L and 50μ L, respectively. The content to be transferred from dilution well to antibody coated well remains the same as 100μ 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:

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. <u>Make sure that the linearity coefficient (r^2) of the calibration curve is no less than 0.985</u>. 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 a flatoxin 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 I	nnovation Lab		
Afghanistan			
Title: Sample Preparation and Test Procedures for Aflatoxin			
Commodity: Wheat			
Test: AFLA Assay 4/40 (COKAQ1000/COKAQ	21048)		
Written by: Luis Sabillón	Edited by: Andréia Bianchini		
Effective date: 05/11/2015	Version: 1		

Sample Preparation:

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

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 <u>Disposal of</u> <u>Samples.</u>

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.

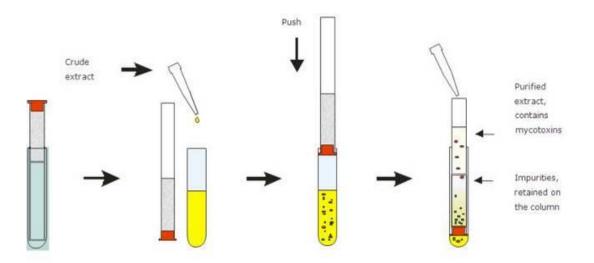


Figure 1. Procedure for using the MycoSep 112 columns

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 μ L/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 μ L of Conjugate into each blue/green-bordered Dilution Well.
- 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 μ L/well or 1 mL/strip) and dispense into a separate container (e.g. reagent boat for an 8- channel pipettor). Pipette 100 μ L 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 μ L/well or 1 mL/strip) and dispense into a separate container (e.g. reagent boat for an 8-channel pipettor). Pipette 100 μ L 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 100μ L and 50μ L, respectively. The content to be transferred from dilution well to antibody coated well remains the same as 100μ 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:

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. <u>Make sure that the linearity coefficient (r^2) of the calibration curve is no less than 0.985</u>. 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 a flatoxin 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: Luis Sabillón	Edited by: Andréia Bianchini		
Effective date: 05/11/2015	Version: <u>1</u>		

Sample Preparation:

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

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 <u>Disposal of</u> <u>Samples.</u>

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 $20-24^{\circ}C$ (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.

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- 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 μ L 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 <u>AgraVision Reader</u> 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)			
			48)
			Edited by: Andréia Bianchini
			Version: <u>1</u>
r r			

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.

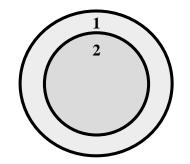


Figure 1. Procedure for reducing sample size.

- 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 μ L/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 μ L of Conjugate into each blue/green-bordered Dilution Well.
- 4. Using a single channel pipettor, add 100 μ L of each standard or sample into the appropriate Dilution Well containing 200 μ L 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 μ L/well or 1 mL/strip) and dispense into a separate container (e.g. reagent boat for an 8- channel pipettor). Pipette 100 μ L 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 μ L/well or 1 mL/strip) and dispense into a separate container (e.g. reagent boat for an 8-channel pipettor). Pipette 100 μ L 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 100μ L and 50μ L, respectively. The content to be transferred from dilution well to antibody coated well remains the same as 100μ 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:

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. <u>Make sure that the linearity coefficient (r^2) of the calibration curve is no less than 0.985</u>. 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 In	novation Lab	
Afghanistan		
Title: Sample Preparation and Test Procedures for Ochratoxin		
Commodity: Wheat		
Test: OTA Assay 2/40 (COKAQ2000/COKAQ20	948)	
Written by: Luis Sabillón	Edited by: Andréia Bianchini	
Effective date: 05/11/2015	Version: <u>1</u>	

Sample Preparation:

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

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 <u>Disposal of</u> <u>Samples.</u>

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 μ L of each standard or sample into the appropriate Dilution Well containing 200 μ L 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.
- 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 μ L/well or 1 mL/strip) and dispense into a separate container (e.g. reagent boat for an 8-channel pipettor). Pipette 100 μ L 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 100μ L and 50μ L, respectively. The content to be transferred from dilution well to antibody coated well remains the same as 100μ 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 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. <u>Make sure that the linearity coefficient (r^2) of the calibration curve is no less than 0.985</u>. 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 Innovatio	on Lab	
Afghanistan		
Title: Sample Preparation and Test Procedures for T2		
Commodity: Wheat		
Test: T2 ASSAY 20/500 (COKAQ6000/COKAQ6048)		
Written by: Luis Sabillón	Edited by: Andréia Bianchini	
Effective date: 05/11/2015	Version: <u>1</u>	

Sample Preparation:

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

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 <u>Disposal of</u> <u>Samples.</u>

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 and 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 ml/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 μ L of each standard or sample into the appropriate Dilution Well containing 200 μ L 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 ul of contents from each Dilution Well into a corresponding Antibody Coated Microwell.
- 6. Incubate at room temperature for 10 minutes.

Note: Don 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 μ L/well or 1mL/strip) and dispense into a separate container (e.g. reagent boat for an 8- channel pipettor). Pipette 100 μ L 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 (\sim 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 100μ L and 50μ L, respectively. The content to be transferred from dilution well to antibody coated well remains the same as 100μ 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 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. <u>Make sure that the linearity coefficient (r^2) of the calibration curve is no less than 0.985</u>. 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.







PHL Innovation Lab		
Afghanistan		
TITLE: Procedure for Decontaminating and Disposing Materials used During Mycotoxin Analysis		
Written by: Luis Sabillón	Edited by: Andréia Bianchini	
Effective date: 05/11/2015	Version: <u>1</u>	

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	Edited by: Andréia Bianchini	
Effective Date: <u>05/11/2015</u>	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 "<u>advisor</u>" 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: <u>06/29/2015</u>	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

PHL Lab's Summary of Information on the MoCI/Raisin Institute Laboratories





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." Moquamuddin 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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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

 ANDS Afghanistan National Development Strategy ANPP Afghanistan National Priority Program ANSA Afghan National Standards Authority 	
ANSA Afghan National Standards Authority	
ANSF Afghanistan National Strategic Framework	
DAIL(s) Directorate of Agriculture, Irrigation and Live	stock
E. coli Escherichia coli	
FASForeign Agriculture Service	
GAIN Global Agricultural Information Network	
GAP Good Agricultural Practices	
GIROA Government of the Islamic Republic of Afgha	nistan
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 Liveston	ck
MoCI Ministry of Commerce and Industry	
MoPH Ministry of Public Health	
MT Metric Ton	
MY Marketing Year	
NADF National Agricultural Development Framewo	rk
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 expediential 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 sanitarily 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						
Inventary #	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						
Inventary #	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

PHL Lab's Summary of Information on the MoCI/Raisin Institute Laboratories

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	1	Asia Sci	4/10/05	
USAIDRAMP0043	Water Still WI460	1	USA	İ	Asia Sci	4/10/05	
	Hand Sucking Pumps	3			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	110011	Asia Sci	4/10/05	
	Burner By 400	2	Pakistan		Asia Sci	4/10/05	
USAIDRAMP0045	Colorimeter Series	1	T akistari	NV201	Asia Sci	4/10/05	
USAIDRAMP0045	Ph Meter	1	Romania	373593	Asia Sci	4/10/05	
USAIDRAMP0040	Refractor Meter Indust.	3	Nomania	373393	Asia Sci	4/10/05	
USAIDRAIVIP0047		3					
	Hot Plate			000500	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			Asia Sci	4/10/05	
	100ml Pyrex Beaker	6			Asia Sci	4/10/05	
	250ml Pyrex Beaker	6			Asia Sci	4/10/05	
	500ml Pyrex Beaker	66			Asia Sci	4/10/05	
	1000ml Pyrex Beaker	6			Asia Sci	4/10/05	
	3000ml Pyrex Beaker	3	Pakistan		Asia Sci	4/10/05	
	Solution Bottles	12			Asia Sci	4/10/05	
	500ml Reagent Bottle	24			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	Γ	Asia Sci	4/10/05	
USAIDRAMP0052	Vacuum Pump	1	China		Asia Sci	4/10/05	
	Filter Paper	3box	China	1	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	1	Asia Sci	4/10/05	
	spectrophotometer	1	China	1	Asia Sci	4/10/05	

Chemical Reagents						
Inventory #	Description	Quantity	made in	Serial No	Transferred	
Sodium Chloride	2Kg	India		Narang	5/10/05	
Na2HPO4	1Kg	India		Narang	5/10/05	
KH2PO	1Kg	India		Narang	5/10/05	
KCI	1kg	India		Narang	5/10/05	
HCI	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	
KmNO4	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	

Table 3

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)
- <u>Rapid Fluorometer Detection</u> 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.

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

Table 4

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 flourometeric method, and preventiative 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

Strengths	Weakness
 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. 	 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.
Opportunities	Threats
• Create a core group of Food Safety Specialist by educating 10 MS and PHDs in food science.	• 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 basics causes of postharvest loses 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 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. Agaragar and dextrose.

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 MIDIUM	24	Functioning
16	TEST TUBE LARGE	24	Functioning
17	MAGNIFING LENS	6	Functioning
18	SLANDER DIFF	6	Functioning
19	FLASK	6	Functioning
20	BEAKER	12	Functioning
21	Protective cloths (complete set)	12	Functioning

Equipment available in the PPQD labs

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.

Name	Job title	Purposes of the meeting
		Discussion establishment of
		Mycology laboratory, training,
	Head of Plant Protection and	certifications and maintenance
Mr. Gurbandi	Quarantine Departments	system
		Discussion on technical aspect and
	Deputy/ admin of Plant	management of quality control
Mr. Tahir	Protection and Quarantine	laboratories
		Discussion on technical aspect and
		management of quality control
Abdul Ghafoor Baburi	Head of Diagnostic Lab	laboratories
	<u> </u>	Discussion about qualification,
		experience, skills regarding routine
Mohammad Nasir		tests and needs of more technical
Ebrahimkhail	Insect pest specialist	support.
	• •	Discussion about qualification,
		experience, skills regarding routine
		tests and needs of more technical
Jamaluddin Stankzai	Insect Ecologist	support.
		Discussion about qualification,
		experience, skills regarding routine
		tests and needs of more technical
Abdul Wasi Hakimi	Seed pathologist	support.
		Discussion about qualification,
		experience, skills regarding routine
		tests and needs of more technical
Patoni Azizi	Biological control assistant	support.
		Discussion about qualification,
		experience, skills regarding routine
		tests and needs of more technical
Ejaz Ahmad	Consultant- Biological control	support.
		Discussion about qualification,
		experience, skills regarding routine
		tests and needs of more technical
Qudratullah Soofizada	Change management specialist	support.
		Discussion about qualification,
		experience, skills regarding routine
		tests and needs of more technical
Mirwais Khogyiani	Change management specialist	support.

Key meeting with following PPQD staff

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 in 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 loose 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 strengthen 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
Asiabs and Grist Mill Flour of Afghan Origin	104 - 144
Asiabs and Grist Mill Flour of Kazakhstan Origin	5 - 9
Asiabs 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,	8
Kabul, Kunduz, and Herat, as recommended by grain	
traders or farmers.	

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 then 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 Amonds	15 - 24
Ghorbandi Alomonds	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	
МоСІ	Ministry of Commerce and Industry	
МоРН	Ministry of Public Health	
MSDS	Material safety data sheets	
МТ	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
- Disposable 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.

#	Name	Designation	Qualificat ion (Level)	Specializati on	Phone	Email
PP	QD Staff					
1	Kh. Aminuddin	Plant Quarantine General Manager	BSc	Plant Protection	(0)7994456 71	<u>kwawajaa4o@yahoo.com</u>
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 Sakhi	Microbiology	MSc	Plant Protection	793645354	aziz-sakhiz-02@yahoo.com

Table 1: List of staffs for the Mycotoxin sample analysis training

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.co m
11	Azim Khan Habib	IPM manager	BSc	Plant Protection	706484101	<u>azimkhanhabibl@gmail.co</u> <u>m</u>
12	Hemayatullah	Mycologist	MSc	Plant Pathology	799707423	hemayatullahrahil@yahoo.c om
13	Mohamad Naser Ibrahim Khail	Entomology - General Manager	BSc	Plant Pathology	799309151	<u>m.naseribrahimkhail@gmai</u> <u>l.com</u>
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)7803572 91	-
20	Mr. Zakria Faizi	Lab Tech		Plant Protection		-
DAI	L 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: Conducing 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.

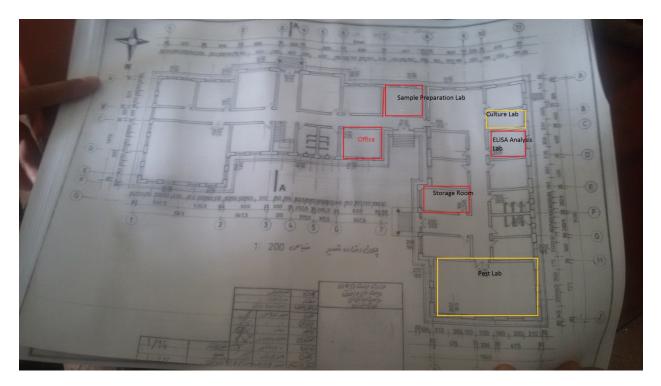


Figure 5: Blueprint of the PPQD laboratory in Kabul, showing specific areas occupied by the mycotoxin laboratory.

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.

Type of Samples	Number of Samples	
Asiabs and Grist Mill Flour of Afghan Origin	104 - 144	
Asiabs and Grist Mill Flour of Kazakhstan Origin	5 - 9	
Asiabs 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,	0	
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	

Table 2: Proposed Wheat / Flour / Wheat Products Sampling Scheme.

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 then 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 Amonds	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.

				Sample	s of dried fru	uits/nuts, wh	eat and whe	at products	collected in	Mazar							
		Raisin				Almond					Pista	chios			Wal	nuts	
Type of sample	Sun dried Tayefe Raisin (rose colored)	Other Raisin	Sattarbai (soft-shell) Almonds	Shokorbai Hard-shell Almonds (5- 8) A02	Wahidi (hard-shell)	Qambari (hard shell & exported) Almonds	Kherudini	Qaharbai	Ismaller	(closed mouth)	(with skins or Shuli)	safid (onen	Other varieties of	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		2	. 1	. 1	1	
Total Sample		14				25						2				6	
			14/	heat and Wh													

			W	heat and Wh	neat product	s		
Type of Sample	Asib Mill	Grist Mill	Flour of Kazakhstan Origin (5)	Grist Mill Flour of Uzbekistan	Improved	or storage	Naan	Commercial Flour Mill
ample 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 o	f dried fruits/n	uts, wheat a	nd wheat pr	oducts colle	cted in Heart					
		Raisin			Alm	ond			Pistachios		Wal	nuts
Type of sample	Quality round	Medium Quality long green seedless raisin	Other raisin	Abdul Wahidi (hard-shell) Almonds	Sangaki and Murawaji Almonds (smaller kernels)	Other Almond	Almonds (smaller	Korak (closed mouth) Pistachios	(with skins or Shuli)		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			1	1			9			6

			Wheat and Wh	heat product	S					
Type of sample and	Asiab Mill	Grist Mill	Asiabs and Grist Mill Flour of Kazakhstan Origin (5) W03	Grist Mill Flour of Turkmanista	Grist Mill Flour of	Purdue Improved Crop Storage (PICS) bags	Cmmercial 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	25			
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.



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; 36/181samples 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

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note peak area - no quantitati same set of 650 metabolites; a used for spiking experiments orksheet is below the limit of detection in all samples

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ഞ്ഞാ. അതിനെ അറമന്ത്രം നിന്ത്രം അറമന്തരം അറമന്തരം അറമന്തരം അറമന്തരം അറമന്തരം അറമന്തരം അറമന്തരം അറമന്തരം അറമന്തര അറമന്മെറ്റ് പറ്റെ അറമന്തരം അറമന്തരം അറമന്തരം അറമന്തരം അറമന്തരം അറമന്മെന്തരം അറമന്തരം അറമന്തരം അറമന്തരം അറമന്തര അറമന്തരം അറമന്തരം അറമന്തരം അറമന്തരം അറമന്തരം അറമന്തരം അറമന്തരം അംഭവ്വാനം അറമന്തരം അറമന്തരം അറമന്തരം അറമന്തരം അ	<ldd (100="" 0.16="" 1106.00="" 19900000="" 42990.00="" 9.02="" 90.16="" 991.16="" <="" <ldc="" <ldd="" ldd="" p=""></ldd>	
	<td></td>	
	- xunc < xunc < tunc < tunc < tunc < 100 31730000 4LCD 1288100 4LCD 1358000 4LCD 1358000 4LCD 1358000 4LCD 4LCD 1358300 4LCD 100 4LCD 1358300 4LCD 4LCD 100 100 100 4LCD 4LCD 100 4LCD 100 4LCD 100 4LCD 4LCD 100 4LCD	
	<ldd 188000="" 1880000="" 188100="" 2282000="" 31470="" <="" hdd="" hdd<="" ldd="" td=""><td></td></ldd>	
	<100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <100 <10	
	LUD 4.00 4.00 4.00 5.00 5.00 1100000 4.00 4.00 4.00 4.00 5.00 5.00 5.00	
	<100 L65 L65 <100 <1700000 <100 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <1000 <10	
	xuut ≤ LUD < LUD < LUD ≤ LUD 9120000 0LDD 916100 12000 1200 1998.00 0LDD 1998.00 0LDD 1998.00 0LDD 12980.00 0	
	<cud (100="" -lod="" 0.40="" 0400="" 0400<="" 0500="" 11110803="" 151100="" <lod="" clod="" td=""><td></td></cud>	
	<100 1.07 (100 (100 (100 000)) 100000 (100 (100 (
	< LDD 10.47 0.52 <ldd 20070.00="" 30070.00="" 3474000.00="" 3844.00="" <="" <ldd="" p=""></ldd>	
number origination origination <thore< th=""> orif origination<td>4 118 40 41 58 203 57 117 57 156 2.0 67.6 29.4 20.1 22.4 99.5 27.9 57.4 27.9 76.5 2.96 1.32 0.49 1.05 1135.79 5.35 155.11 2.92.66 6.13 4440.03</td><td>Market America Second Second</td></thore<>	4 118 40 41 58 203 57 117 57 156 2.0 67.6 29.4 20.1 22.4 99.5 27.9 57.4 27.9 76.5 2.96 1.32 0.49 1.05 1135.79 5.35 155.11 2.92.66 6.13 4440.03	Market America Second Second

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

| Date of File
 | Random
Namber | Sample 10
 | Currentedity
 | Province
 | Type of Sample | Tampling Location | Caurity | CPS Location
 | Individual 3 | iumple log | Sampling | androiLag | Tise Cont | cool Sag | pH
Alghanistan
 | Mp | atacin Analysis Pe | formed in Alghar | isten | Alghanistan | UNL
 | LINE. | Audria | Autola |
|---
---|--

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---|--|--|---
--|---|------------|---|-----------|---|----------
---|--|--------------------|------------------|-------------
---|--
---|---|---|
| alits/2005 15
 | | Sample 10
 | Commodity
Almond - ADS
 | Paulina
 | Type of Sample | Dry Truit Market, Maar | Cauntity | CPS Location
N (6), 62, 2, 1299 167, 6, 66, 3600
 | Tes
X | No. | Tes
X | No | Tes
X | No | Alghanistan
7.27
 | Aliatonia
X | DON | N | Ochristanin | Aflationin ppb
<lod< th=""><th>Aflabouts ppb
EIIC</th><th>Affatosis ppb</th><th>Aflatanin pyb
<100</th><th>Ochroteoire pyb
< 1000</th></lod<> | Aflabouts ppb
EIIC
 | Affatosis ppb | Aflatanin pyb
<100 | Ochroteoire pyb
< 1000 |
| 555
 | 461 | AD-99-10-10-29-2010-010
AD-99-10-10-29-2010-089
 | Almond - ADS
Almond - ADS
 | Parwan
Parwan
 | Ghadaad Alomandi
Sattada tahuhat Alomandi | | 10
10 |
 | | | | | | |
 | | | | | | Train
Train
 | <100 | (100 | <100 |
| 0
 | 465 | AD 532 10 ID 2015 002
 | Almond - AE2
 | Samangan
 | Satadas Sofi-Dell Amando
Shokarka Hará-del Almondo | | |
 | | | | | | |
 | | | | | | <100
 | <100 | <100 | <500 |
| 8/26/2025 13
 | | AD-012-015-023-023
 | Almond - ADS
Almond - AD7
 | Balkh
Sanangan
 | Abdul Walnuk Almonds.
Other Almonds | Dry Null Market, Micar | 10
10 | N 94,42,2,880
 | ж | | × | | * | | 7.65
 | ж | | | | <100 | <100
 | <100 | <100 | <100 |
| 9/2/2015 12
 | 468 | A25 H 12 8 9 2015 012 001
 | Almond - AET
 | Heat
 | Other Almonds | Bank d., Herat | " | N 94,20,89,4600
 | х | | х | | | |
 | | | | | | <100
 | <100 | <100 | <100 |
| 16/26/2028 118
8/2/2028 2
 | 409 | 825 9 53 9 9 15 02 02
829 98
 | Almond - AET
Almond - AEE
 | Raho
Kabul
 | Other Almonds
Qamkari Amonds (very strong almond flavor) | Naji Mil Hachevi dry fruit market, Matar
Gabul - Mandawi | 10
10 | N 39,42,39,480 167,4,47,7000
N 34,33,35,4699
N 34,33,35,4699
 | x | | x | | x
x | | 7.66
 | x | | | | 7.85 | <100
 | <100 | <100 | <100 |
| 9/2/2015 25
20/29/2015 867
 | 473
472 | A 26 & 55 & 6 2713 - 675 - 671
 | Almond - ADS
Almond - ADS
 | Rahin
Kabul
 | Sangaki and Marawaji Almonds (analierkennels)
Sattaskai Soft-shell Almonds | Juma Chan dry fruit market, Micor
Kabul, Mandawi | 10
10 | N 36(42),5,29800
1873 6,473,899
N 34(30),53,5399
 | x | | x
x | | x | | 4.39
 | x | | | | <100 | 5.85
 | <1200
125.25 | <1000
12.78 | <100
<100 |
| 10/23/2005 805
 | 471 | A25 406-51-10-3-13-825-020
 | Almond - ADT
 | Kabul
 | Other Almands | Kabul - Mahdawi | " | N 54,53,25,2999
5556,52,2999
 | х | | x | | ж | | 2.73
 | ж | | | | < 100 | <100
 | < 600 | < 600 | <500 |
| 10/29/2005 X78
9/2/2015 X8
 | 471
475 | AD-681-12-12-0-12-129-029
 | Almond - AEL
Almond - AET
 | Kabul
Heat
 | Sattarbai Soft-shell Almonds
Other Almonds | Kabul, Mandawi
Kabulo - Khadi, Herat | 1)
1) | N 14, 30, 34, 6000
N 14, 20, 37, 2399
 | x | | x | | * | | 631
 | х | | | | < 600 | <100
 | <100 | <100 | <100 |
| 10/27/2028 847
8/10/2028 20
 | 675
677 | A25-681 43-33-6 35-38-688
A25-6-53-6-53-680 488
 | Almond - AET
Almond - AES
 | Kabul
 | Other Almands
Sattarkai Soft-shell Almonds | Gibal, Mandawi | " | N 54,80,56,0800
 | ж | | x | | ж | | 7.85
7.85
 | ж | | | | < LOD
7.36 | <600
 | <100 | <100 | <100 |
| 8/10/0015 10
40
 | | A20-9-12-12-29-2019-000-
 | Almond - AEL
 | Bahih
Samangan
 | Sattadus Soft-diell Almonds
Sattadus Soft-diell Almonds | Naji Muhammad Kibar diy Sult market, Masar | " | N 16(42), (2999 187,6(46,6000
 | | x | x | | x | | 2.05
 | x | | | | 7.% | <100
1079
 | <100 | <100
<100 | <100
<100 |
| 90
10/23/2005 806
 | | 820-5-10-10-10-2015-080
825-681-10-10-5-10-308-029
 | Almond - ADS
Almond - AD7
 | Samangan
Kabul
 | Abdul Walked Almonds
Other Almonds | Kabul - Mandawi | " | N3430,25,299
 | х | | x | | ж | | 2.47
 | х | | | | < LOD | 5.11
<100
 | <100 | <100
<100 | <100 |
| 250
 | 45 | A32-6N-53-0-9-15-250-012
 | Almond - A22
 | Kadahar
 | Shokashai Hard-chell Almonds | | " |
 | | | | | | |
 | | | | | 11.34 | <100
 | < 600 | <100 | <100 |
| 10/29/2018 117
9/5/2015 20
 | 463 | A 20 401 - 12 - 12 - 13 - 13 - 12 - 220
A 27 - 0 12 - 0 - 13 - 220 425
 | Almond - ADS
Almond - AD7
 | Kabul
Baha
 | djambast Amonds (very strong atmond flavor)
Other Atmonds | Kabul - Mandawi
Dry Nuit Market, Macar | 2 | N 36,42,2,7020 167,6,48,489
 | x | | x | | x | | 7.52
 | x | | | | 199 | 5.85
 | <100 | <100 | <100 |
| 10/27/2018 807
 | 481 | 830-681-12-10-1-12-327-028
830-681-12-10-0-13-382-027
 | Almond - ADS
Almond - ADS
 | Kabul
Kabul
 | Gambari Amonds (very strong almond flavo)
Sangati and Murawaji Almonds (anatier kennels) | Kabul - Mindawi
Kabul, Mindawi | " | N 14, 30, 25, 2999
N 14, 30, 25, 2999
N 14, 30, 25, 5200
 | x | | x | | x
x | | 6.67
7.09
 | x | | | | 1949
< 100 | <100
 | Tike
<100 | <100 | <100 |
| 481
 | 488 | AZI-99-13-10-28-2013-480
 | Almond - AEL
 | Farwan
 | Sattarka: Soft-shell Almonds | | " | NIN 62-06-300
 | | | | | | |
 | | | | | | <100
 | <100 | <100 | <500 |
| #/10/2005 45
 | 468 | 825-9-52-9-15-025-026
825-9-52-9-32-025-0268-026
 | Almond - AES
Almond - AES
 | Baltiti
Herat
 | Sattañai Sofi-chell Almonds
Abdul Wahod Almonds | Dry fruit market of Mil. Brahan, Mazar
Arab Kandahar Azimi Mandawi, Herst | " | N 16(-02)-66, 3600
N 16(-20), 8, 8800
N 16(-20), 8, 8800
 | x | | x | x | x
x | | 7.88
 | x | | | | 33.86
Trace | 549.21
 | 224.25
<1020 | MR.45
5.81 | <100 |
| 10/23/2005 234
8/26/2005 84
 | | A 20-KN 42-9-9 15-230-015
A 20-H 50-9 12-2015-0280-001
 | Almond - ADS
 | Kandahar
Heigt
 | Sangaki and Marawaji Almonds (analierkennets)
Abdul Wahod Almonds | Radan Sherkat Market, Kandahar | " | NTL 14, 47 APR 148, 49, 28, 490
 | x | | х | × | x
x | | 6.51
7.83
 | x | | | | 548.27
< LOD | <100
 | <1000
6.25 | <100 | <1000
<1000 |
| 10/29/2015 447
 | 495 | AZI-486-51-10-11-15-867-030
 | Almond - AES
 | Kabul
 | Satta-bai Soft-chell Almonds | Kebul, Mandaei | " | N14,10,11,1099
 | ж | | х | - | ж | | 7.25
 | х | | | | < 100 | <100
 | <100 | <100 | <100 |
| 8/80/3005 X8
30/29/2005 485
 | 610
611 | 830 8 53 6 9 15 088 008
835 686 10 10 10 15 485 004
 | Almond - ADS
Almond - AD2
 | Rahah
Kabul
 | Quelant Amonds (very strang almond flavor)
Shokashar Hard-shell Almonds | Nophuliah Dry Suit Market, Masar
Kabul, Mandawi | 10
10 | N 84,42,48,4800
N 84,80,34,0700
 | x | | x | | x
x | | 7.55
 | x | | | | < LOD
5.35 | <100
 | <100 | 0.90
<1200 | <100
<100 |
| 10/23/2028 888
 | 494
495 | A25-601-11-10-1-10-000
A25-5-11-10-01-2015-000
 | Almond - AET
 | Kabul
 | Other Almands | Gibal, Mandawi | " | N 54, 52, 52, 59, 8999
 | х | | х | | х | | 4.89
 | х | | | | 6.95 | <100
 | <100 | <100 | <100 |
| #/XL/0005 62
 | | A25-9-10-00-2023-080-
A26-9-50-8-7-05-082-002
 | Almond - AD6
 | Samangan
Herat
 | Sataka Sufi-oleit Amonds
Sangaki and Murawaji Almonds (unaller kernels) | Arab Kandahar Azimi Mandawi, Herat | 10
10 | N9420,412999
 | ж | | x | | ж | | 7.6
 | ж | | | | < 100 | 1009
<100
 | (100 | <100 | <000
<000 |
| 10/23/2005 338
9/2/2005 11
 | 687
698 | A 26-481-12-22-3-23-238-22.7
 | Almond - ADS
 | Kabul
 | Sangaki and Marawaji Almonds (analier kennels)
Gambari Amonds (very strang almond flavor) | Kabul, Mandael
Div Nutl Madel, Micar | " | N 54, 82, 93, 87, 99
646 5 5 66 69
N 26 62 2 199 28 7 6 33 (2009
 | x | _ | x | | х | | 7.28
 | х | | _ | | 500 | <100
 | <100 | <100 | <100 |
| 500
 | 499 | AID-98-35-10-28-2019-500
 | Almond - A21
 | Parwan
 | Satta-bai Soft-chell Almonds | | " | 100,000 00,000
 | | | | | | |
 | | | | | | <100
 | <100 | <100 | <100 |
| 81
11/14/2025 568
 | | AD 510 10 10 10 2015 00
AD 601-10 10 10 10 10 10 10 10
 | Almond - AE2
Almond - AE6
 | Samangan
Kabul
 | Shokarbai Haril-shell Almonds
Qambail Amonds (very strang almond flavor) | Kabul, Mendawi | 10
10 | Lie IN, OKENNES
 | x | | x | | ж | | 4.99
 | ж | | | | 12.88 | <100
535
 | 11809
<1000 | <100 | <100 |
| 10/21/2028 811
UNL 72
 | 502 | A25 486 43 43 43 43 43 43 43
 | Almond - AET
Almond - AES
 | Kabul
Samangan
 | Other Almands
Sattarkai Soft-shell Almands | Kabul - Mandawi | 10
10 | N 54,83,25,2999
5576-52,2999
 | ж | | x | | ж | | 6.75
 | ж | | | | 5.52 | 500
<100
 | <100 | <100
<100 | < 100
< 100 |
| 10/23/2005 880
 | 504 | A20 KHL-51-10 3-13-33D-82H
 | Almond - ADS
 | Kabul
 | Gambas Amonds (very strong almond flavor) | Golul, Mindaei | " | N 94,30,56,5300
899,20,36,999
 | х | | x | | x | | 7.38
 | х | | | | 13.60 | <100
 | <000 | <100 | <500 |
| au
73
 | | AD 99 10 10 10 21 210 40
 | Almond - ADS
 | Parwan
Samangan
 | Abdul Walna Almonds
Sattarbai Soft-shell Almonds | | " | | | |
 | | | | | | |
 | | | | | |
 | | <100 | <100 |
| UNL 64
 | | A36-5-12-12-10-2013-060
 | Almond - ADS
 | Samangan
 | Sangaki and Murawaji Almonds (unaffer kemels) | | " |
 | | | | | | |
 | | | | | | <100
 | <100 | <100 | <100 |
| UNL 202
UNL 48
 | 529 | A20-03-12-0-9-2233-282-
A20-5-12-12-10-2233-088-
 | Almond - ADS
Almond - ADS
 | Kandahar
Samangan
 | Sangaki and Marawaji Almonds (analiter kernels)
Abdut Wahod Almonds | | 2 2 |
 | | | | | | |
 | | | | | | <100
Tala
 | <100 | <100 | <100 |
| 8/30/3005 4
8/25/3005 6
 | 510 | AZI-0-54-0-15-022-022
AZI-0-53-0-2223-05-002
 | Almond - AEL
Almond - AEL
 | Rafillo
Rafillo
 | Sattañai Sofi-chell Almonds
Shokarbai Hard-chell Almonds | Naji Mé Hadam Dry frat Market Macar | 11
11 | N 36/42/3, 6320 367/6/47, 1999
 | x | | x | | ж | | 7.6
 | х | | | | 22.33 | <100
 | < LOD
Tikke | <100 | <100 |
| 63
 | 512 | A20-3-12-12-02-2013-089
 | Almond - AEL
 | Samangan
 | Sattarkai Soft-shell Almonds | | | | | |
 | | | | | | |
 | | | | | |
 | | <100 | <100 |
| 8/80/2005 22
UNL 485
 | | A 20 0 51 0 0 15 022 021
A 20 99 10 10 10 20 2215 485
 | Almond - ADS
Almond - ADS
 | Ballah
Panwan
 | Qambail Amonds (very strang almond flavor)
Sattabail Soft-chell Almonds | Bry Null Market of Hop Muhammad Akbar,
Miscos | 10
10 | N 39,42,2,666 557,6,48,1800
 | х | | x | | ж | | 2.96
 | х | | | | <100 | <100
 | <udd
Tikke</udd
 | <100 | <100 |
| UNL 505
UNL 53
 | | A20-99-10-10-29-2010-500-
A20-9-10-10-20-2010-000-
 | Almond - ADS
 | Forward
 | Abdul Walkoll Almonds
Tangali and Murawaji Almonds (analisr kernets) | | " |
 | | | | | | |
 | | | | | | <100
 | <100 | <100 | (100 |
| UNL 477
 | 312 | AD 99-10-10-10-2010-077
 | Almond - ADS
Almond - ADS
 | Sanangan
Parwan
 | Abdul Wahidi Almonds | | 10
11 |
 | | | | | | |
 | | | | | | <100
 | <100 | <100 | <100 |
| 10/23/2005 230
10/23/2005 387
 | | 836-03-12-0-0-12-230-016
830-081-12-10-0-13-130-010
 | Almond - ADS
 | Kandahar
Kabul
 | Sangaki and Murawaji Almonds (unaller kennels)
Qambari Amonds (very strang almond flavo) | Roser + Muchtanik, Kandobar
Kabul, Mandawi | 10
10 | N 81,84,90,729 148,40,27,278
N 84,30,30,8799
 | x | | x | | x | | 4.83
 | x | | | | < LOD
5 GAR | <100
 | <100
<100 | <100
<100 | <100 |
| 8/80/2005 18
 | 120 | A36 ++ 12 +0 7 13 -029 025
 | Almond - AD6
 | Heat
 | Sangaki and Murawaji Almonds (analier kennels) | Carvao + Kharih, Herst | " | N34,20,26,4799
 | ж | | х | | x
x | | 2.3%
 | ж | | | | < LOD |
 | | <100 | <000 |
| 8/80/2015 78
10/27/2015 888
 | | A20 ++ 50 @ 13 13 CT# 027
 | Almond - ADS
 | Heat
 | Abdul Wahed Almonds | recit - Taskeed Market | 10 | Las in accession
 | ж | | × | | | | 7.73
 | ж | | | | |
 | < 100 | < 600 | <100 |
| 8/28/2005 5
 | | A26-KHL-12-12-0-13-380-012
 | Almond - AD6
 | Kabul
 | Sangaki and Marawaji Almonds (analier kennels) | Kabul, Mandawi | " | N34,82,54,5399
 | ж | | x | | x | | 4.73
 | ж | | | | 21.36
1040 | <100
 | <100 | <100 | < 600 |
| 10/00/2000 200
 | 523 | A22 0 13 0 11 025 022
 | Almond - ADS
Almond - ADD
 | Earlin .
 | Shekarba Hard-chell Almonds | Dry fruit Market Micar | | N 34,30,54,5399
N 34,42,5,4 800 187,6,47,1999
N 34,30,25,2999
 | x | | x
x | | x
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			Sample ID Commodity Province					1	Individual	ample Log Sampling	antesi ing E	lisa Control Log		Musedonia Applais I	Performed in Afghanist		Afghanistan	UNL	UNL	Austria	Austria	
Date of File	Seq. Number	Random Number	Sample ID	Commodity	Province	Type of Sample	Sampling Location	Country	GPS Location	Ver	No Yes	No Ye	1	pH Afghanistan	Aflatoxin DON	1 1	Orbratosin	Romer Aflatoxin ppb	Romer Aflatoxin ppb	Neosen Aflatoxin ppb	Aflatoxin ppb	Ochratoxin A ppb
10/28/2015	392	600	P03-K8L-S1-10-4-15-392-003	Pistachio - P03	Kabul	Khandan-e-safid Pistachios (strong flavor and	Kabul, Mandawi	AF	Lat: 34,30544600	Tes X	ND Tes	NO 11		7.11	X DON	1-2	Donratokin	8.90	<lod< th=""><th>< LOD</th><th>< LOD</th><th><lod< th=""></lod<></th></lod<>	< LOD	< LOD	<lod< th=""></lod<>
	94	601	P02-S-S1-10-31-2015-094-	Pistachio - P02	Samangan	wrinkly shell Pushdara Pistachios (closed shell with purple	ABOU, Mandawi	AF	Long: 69.10345299										<100	< LOD	< LOD	< LOD
	98	602	P02-S-S1-10-31-2015-098-	Pistachio - P02	Samangan	outer skin) Pushdara: Pistachios (closed shell with purple		AF											<10D	< LOD	< LOD	<100
10/28/2015	457	603	P01-K8L-S1-10-11-15-457-007	Pistachio - P01	Kabul	outer skin) Korak Pistachios (open shell with purple	Kabul, Mandawi	AF	Lat: 34,30552599	×	×		ĸ	7.19	x			5.94			<100	<100
10/28/2015	456	604	P01-KBL-S1-10-11-15-456-005	Pistachio - P01	Kabul	outer skin) Korak Pistachios (open shell with purple	Kabul, Mandawi	AF	Long: 69.10352900 Lat: 34,30551799	×	×		ĸ	7.37	x			115.56	12.17	13.13	1189.13	< LOD
10/20/2015	93	605	P02-5-52-10-31-2015-093-	Pistachio - P02	Samangan	outer skin) Pushdara: Pistachios (closed shell with purple	Kabul, Mandawi	AF	Lone: 69.10354700	^	^		·		^			11.00	570.84	576.88	1120.50	2.52
10/28/2015	445	605	P01-K8L-S1-10-11-15-445-003	Pistachio - P01	Kabul	outer skin) Korak Pistachios (open shell with purple	Kabul, Mandawi	AF	Lat: 34,30538899	×	×		ĸ	7.86	x			18.12	8.61	6.25	4.93	<100
10/28/2015	432	607	P01-KBL S1-10-10-15-432-004	Pistachio - P01	Kabul	outer skin) Korak Pistachios (open shell with purple	Kabul, Mandawi	AF	Lone: 69.10352200 Lat: 34,30538899	×	×		ĸ	7.53	x			<100	<100	< LOD	1.35	<100
10/20/2013	149	608	P02-5-51-11-2-2015-149-	Pistachio - P02	Samangan	outer skin) Pushdara Pistachios (closed shell with purple	NEUL MEILEN	AF	Lone: 69.10352200	^	^			7.66	^				<100	< LOD	0.48	<100
9/6/2015	40	609	P04-R-53-R-3-15-040-001	Pistachio - P04	Balkh	outer skin) Other varieties of Pistachios	Safi dry fruit market, Mazar	AF	N36;42;5,0400	×	×		×	7.75	×			68.85	(100	100	2.79	< 100
11/17/2015	58	610	P01-5-51-10-30-2015-058-015	Pistachio - P04	Samangan	Korak Pistachios (open shell with purple	san dry froit market, wazar	AF	E67:6:48.01999	×	×		ĸ	7.71	×			<100	<10D	19.88	<100	<100
11/17/2015	76	611	P04-B-SS-8-4-2015-076-020	Pistachio - P04	Balkh	outer skin) Other varieties of Pistachios		TI		×	×		ĸ	7.41	×			<100	<100	< LOD	< LOD	<100
11/17/2015	67	612	P04-8-55-8-4-2015-076-020	Pistachio - P04	Samanean	Korak Pistachios (open shell with purple		TJ AF		×	×		x .	7.41	x			<100	45.49	28.75	141.65	<100
11/1//2015	312	612	P01-3-51-10-30-2015-087-018		Kabul	outer skin) Khandan-e-safid Pistachios (strong flavor and		AF		^	^			1.4	^			<100	40.49 <100	< LOD	0.65	2.55
11/17/2016	79	613	P01-5-51-10-31-2015-079-017	Pistachio - P03 Pistachio - P01	Kabul Samangan	wrinkly shell Korak Pistachios (open shell with purple		AF		×	×			7.81	×			10.90	< 100	< LOD < LOD	< LOD	<100
11/1//2016	96	615	P02-5-51-10-31-2015-096-	Pistachio - P01 Pistachio - P02	Samangan	outer skin) Pushdara: Pistachios (closed shell with ourgle		AF		^	^	,		7.81	*			10.90	Trace	Trace	< LOD	< 100
	97	615	P02-5-51-10-31-2015-096-	Pistachio - P02 Pistachio - P02		outer skin) Pushdara Pistachios (closed shell with purple													<lod< td=""><td></td><td></td><td></td></lod<>			
	97				Samangan	outer skin) Khandan-e-safid Pistachios (strong flavor and		AF												Trace	5.72	< LOD
10/28/2015	18	617	P03-H-54-8-11-2015-0080- P01-B-52-8-3-15-018-009	Pistachio - P03 Pistachio - P01	Herat Balkh	wrinkly shell Korak Pistachios (open shell with purple		AF	Lat: 36.4227699		×		ĸ	7.06	×			8.23	<100	27.13	0.36	< 100
						outer skint Korak Pistachios (open shell with purple			Long: 67,64815199	x									< LOD	6.25		
10/28/2015	421	619	P01-KBL-S1-10-10-15-421-008	Pistachio - P01	Kabul	outer skin) Korak Pistachios (open shell with purple	Kabul, Mandawi	AF	Lone: 69.10351099	x	x		к	7.12	×			<100			< LOD	< LOD
10/28/2015	393	620	P01-KBL-S1-10-5-15-393-012	Pistachio - P01	Kabul	outer skin) Khandan-e-safid Pistachios (strong flavor and	Kabul, Mandawi	AF	Long: 69.10345299	x	x		к	6.93	×			339.36			1880.20	< LOD
10/28/2015	17	621	P03-H-S2-8-9-15-0017-001	Pistachio - P03	Herat	wrinkly shell Korak Pistachios (open shell with purple		AF	Long: 64.114983999 Lat: 34.30541399	x	×		к	7.09	×			10.50			2.83	< LOD
10/28/2015	439	622	P01-KBL-S1-10-10-15-439-0011	Pistachio - P01	Kabul	outer skin)	Kabul, Mandawi	AF	Long: 69.10351400	x	x	1	κ	6.96	x			184.41	< LOD	< LOD	0.81	< LOD
	9	623	P04-B-53-8-3-2015-09-	Pistachio - PO4	Balkh	Other varieties of Pistachios Korak Pistachios (open shell with purple		AF											< LOD	< LOD	< LOD	< LOD
11/17/2015	89	624	P01-S-S1-10-31-2015-089-021	Pistachio - P01	Samangan	nuter drint		AF	Lat: 34.30545700	x	x	1		7.88	x			< LOD	100.18	94.63	14.19	< LOD
10/28/2015	403	625	P01-KBL-S1-10-10-15-403-0010	Pistachio - P01	Kabul	Korak Pistachios (open shell with purple outer skin) Korak Pistachios (open shell with purple	Kabul, Mandawi	AF	Lat: 34,30545700 Lone: 60.10353200	x	x	1	κ	7.04	x			300.09			1.66	< LOD
	74	626	P01-H-S4-8-11-2015-0074-	Pistachio - P01	Herat	outer skin)		AF											Trace	6.25	17.60	< LOD
11/17/2015	148	627	P01-S-S1-11-2-2015-148-014	Pistachio - P01	Samangan	Korak Pistachios (open shell with purple outer skin)		AF	N34:32:13.6600	x	x	1		7.72	x			< LOD				
9/6/2015	75	628	P04-B-S4-8-4-15-075-002	Pistachio - PO4	Balkh	Other varieties of Pistachios Korak Pistachios (open shell with purple	Haji Murad Dry fruit Market, Mazar	AF	N34;52;13,6600 E69:7:31.6499	x	x		κ	7.71	x			30.97	77.28	82.00	82.28	< LOD
11/17/2016	88	629	P01-S-S1-10-31-2015-088-018	Pistachio - P01	Samangan	nuter drin)		AF		x	x		к	7.46	x			< LOD	Trace	Trace	< LOD	< LOD
9/6/2015	75	630	P01-H-S1-8-11-15-075-002	Pistachio - P01	Herat	Korak Pistachios (open shell with purple outer skin)	Herat - Ghafood Market	AF	Lat: 34,204767999 Lone: 62.11879999	x	x		к	7.76	x			129.41			0.67	< LOD
11/17/2015	572	631	P01-KBL-S1-11-10-2015-572-019	Pistachio - P01	Kabul	Korak Pistachios (open shell with purple outer skin)		AF		x	x		к	7.43	x			< LOD			< LOD	< LOD
10/28/2015	8	632	P02-8-52-8-3-2015-008-002	Pistachio - P02	Balkh	Pushdara Pistachios (closed shell with purple outer skin)		AF	Lat: 36,4222999 Lone: 67.64676000	х	x	3	к	7.55	x			23.68	27.37	23.75	13.57	< LOD
UNL	1	633	P02-KBL-52-11-21-2015-001-	Pistachio - P02	Kabul	Pushdara Pistachios (closed shell with purple outer skin)		AF							x				34.77	25.50	2942.44	< LOD
UNL	14	634	P02-88L-S1-11-22-2015-014-	Pistachio - P02	Kabul	Pushdara Pistachios (closed shell with purple outer skin)		AF							x				6.58	< LOD	5.74	< 100
UNL	19	635	P03-KBL-S1-11-22-2015-019-	Pistachio - P03	Kabul	Khandan-e-safid Pistachios (strong flavor and wrinkly shell)		AF							x				5.00	< LOD	1.50	< LOD
UNL	6	636	P02-88L-52-11-21-2015-006-	Pistachio - P02	Kabul	Pushdara Pistachios (closed shell with purple outer skin)		AF							x				Trace	6.25	1.17	< 100
UNL	20	637	P02-KBL-S1-11-22-2015-020-	Pistachio - P02	Kabul	Pushdara Pistachios (closed shell with purple outer skin)		AF							x				6.48	< LOD	< LOD	< LOD
UNL	25	638	P04-KBL-S1-11-22-2015-025-	Pistachio - PO4	Kabul	Other varieties of Pistachios		AF							x				12.95	Trace	0.92	< LOD
UNL	8	639	P04-KBL-S2-11-21-2015-008-	Pistachio - PO4	Kabul	Other varieties of Pistachios		AF							x				< LOD	< LOD	51.81	< LOD
UNL	28	640	P03-KBL-S1-11-22-2015-028-	Pistachio - P03	Kabul	Khandan-e-safid Pistachios (strong flavor and wrinkly shell)		AF							x				941.86	1071.88	< LOD	<100
UNL	16	641	P03-KBL-S1-11-22-2015-016-	Pistachio - P03	Kabul	Khandan-e-safid Pistachios (strong flavor and wrinkly shell)		AF							x				< LOD	Trace	< LOD	< LOD
UNL	24	642	P04-88L-S1-11-22-2015-024-	Pistachio - PO4	Kabul	Other varieties of Pistachios		AF							×				5.00	Trace	4.30	< 100
UNL	23	643	P03-KBL-S1-11-22-2015-023-	Pistachio - P03	Kabul	Khandan-e-safid Pistachios (strong flavor and wrinkly shell)		AF							x				39.62	43.50	< LOD	< LOD
UNL	3	644	P04-KBL-S1-11-21-2015-003-	Pistachio - PO4	Kabul	Other varieties of Pistachios		AF							x				< LOD	< LOD	0.86	< LOD
UNL	7	645	P04-KBL-S2-11-21-2015-007-	Pistachio - PO4	Kabul	Other varieties of Pistachios		AF							x				< LOD	Trace	< LOD	< LOD
UNL	2	646	P03-KBL-S2-11-21-2015-002-	Pistachio - P03	Kabul	Khandan-e-safid Pistachios (strong flavor and wrinkly shell)		AF							x				Trace	< LOD	2.43	< 100
UNL	15	647	P03-KBL-S1-11-22-2015-015-	Pistachio - P03	Kabul	wrinkly shell Khandan-e-safid Pistachios (strong flavor and wrinkly cholfi		AF							x				< LOD	6.25	< LOD	< LOD
9/6/2015	7		P01-B-S3-8-3-15-007-001	Pistachio - P01	Balkh	wrinkly shells Korak Pistachios (open shell with purple	Balkh, Dry fruit Market	AF	N36;42;3,2000	x	×	3	к	7.42	x			145.01				
10/28/2015	19		P03-8-51-8-3-15-019-002	Pistachio - P03	Balkh	outer skin) Khandan-e-safid Pistachios (strong flavor and		AF	E67:6:47.73000 Lat: 36;42127000	x	x	1	ĸ	7.09	x			15.10				
10/28/2015	76		P02-H-S3-8-11-15-0076-001	Pistachio - P02	Herat	wrinkly challs Pushdara Pistachios (closed shell with purple	Herat -	AF	Lat: 34,204747000	x	×	1		6.88	x			13.81				
10/28/2015	77		P01-H-S4-8-11-15-0077-013	Pistachio - P01	Herat	outer skin) Korak Pistachios (open shell with purple		AF	Lat: 36,424201000	x	x	1	ĸ	7.17	x			73.87				
10/28/2015	450		P01-KBL-S1-10-11-15-450-006	Pistachio - P01	Kabul	outer skin) Korak Pistachios (open shell with purple	Kabul Mandawi	AF	Long: 67.526799 Lat: 34,30551799	x	x	3	ĸ	7.3	x			80.20				
						outer skin)			Long: 69.10353999	-	I					1 1			L			

| Date of File | le Seq.
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| | Numbe
 | ıq.
nber

 | Random
Number | Sample ID | Commedity
 | Province
 | Type of Sample Sampling Location
 | Country | GPS Location | Individual Sample Log
Yes No |
Samplin
Yes | g Control Log
No | Elisa Contro
Yes | ol Log pH
No Afghasistan | Myci
Aflatoxin | otoxin Analysis Perfo | med in Afgha
T-2 | | Afghanktan
Romer
Aflatoxin ppb | Afghanistan
Borner
Ochratoxin ppb
 | Austria Austria
Aflatoxin ppb Ochratoxin A p |
| | 492
235
 | 25

 | 257
258 | 802-98-51-10-28-2015-492-
807-KN -53-9-10-2015-225- | Raisins - R02
Raisins - R07
 | Parwan
Kandahar
 | Medium Quality Long Green Seedlers Rakin (Dried in shade
and in mathouse Eichnish Khana)
Son dried Tayler (name for the variety from the
north) & Aklassi frame for the variety from the south)
Condition Tayler (name for the variety from the
Condition Tayler (name for the variety from the
 | AF
AF | | |
 | | | | | | | | |
 | <100 <100 |
| 9/12/2015 | \$\$7
 | 57

 | 259
260 | R07-H-52-8-9-15-010-002
R07-KBL-51-11-10-2015-557- | Raisins - R07
Raisins - R07
 | Herat
Kabul
 | Sun dried Typefee (name for the variety from the Tunhich Market, jada-e-Hear, Mandawi,
north) & Abieux frame for the variety from the such
Sun dried Typefee (name for the variety from the
north) & Abieux frame for the variety from the
Sun dried Typefee (name for the variety from the
 | N
AS | N34(20)39,6900
562-11-27,4200 | × | x
 | | x | 6.56 | × | | | × | <100 | < 100
 | <lod <lod<br=""><lod <lod<="" td=""></lod></lod> |
| | 237
 | 02

 | 261
262 | 807-KN 51-9-10-2015-227-
807-KN 53-9-8-2015-222- | Raisins - RD7
Raisins - RD7
 | Kandahar
Kandahar
 | Sun dried Tayelee (name for the variety from the
needs) Alabana Ensues for the variety from the
Sun dried Tayelee (name for the variety from the
events) & Alabana Ensues for the variety from the
Sun dried Tayelee (name for the variety from the
Sun dried Tayelee (name for the variety from the
 | 45
46 | | |
 | | | | | | | | |
 | <100 <100
<100 <100 |
| | 229
239
 |

 | 263
264 | 807-KN-52-9-9-2015-229-
807-KN-52-9-10-2015-229 | Raisins - R07
Raisins - R07
 | Kandahar
Kandahar
 | Sun dried Tayefee (name for the variety from the
north) & abinue insues for the variety from the exath)
Sun dried Tayefee (name for the variety from the
 | AF
AF | | |
 | | | | | | | | |
 | <lod <lod<br=""><lod <lod<="" td=""></lod></lod> |
| 9/12/2015 | 236
15 7
 |

 | 265
266 | R07-KN-53-9-10-2015-236-
R07-H-51-8-9-15-007-007 | Raisins - R07
Raisins - R07
 | Kandahar
Herat
 | andel R. Advance faces for the variety from the second
E. On fried Tayletic (instruct for university from the
month) B. Advance frame for the variety from the south)
Ean direct Tayletic (name for the variety from the
south) B. Advance for the variety from the south)
Hence
 | AS
AS | N34,20,38,3899
662-11-27,9200 | × | ×
 | | x | 6.88 | × | | | × | <lod< td=""><td>< 100</td><td><lod <lod<br=""><lod <lod<="" td=""></lod></lod></td></lod<> | < 100
 | <lod <lod<br=""><lod <lod<="" td=""></lod></lod> |
| | 581
436
 |

 | 267
268 | R05-KRL-51-11-10-2015-581-
R05-KRL-51-10-10-2015-436- | Raikins - RDS
Raikins - RDS
 | Kabul
Kabul
 | San dried Shomali Rakin (juun dried, black in color, has a
intoar concost ansae Tiware bas anali seeds. Often exported
San dried Shomali Rakin (juun dried, black in color, has a
intoare concost ansae Tiware bas umali seeds. often exported
 | AF
AF | | |
 | | | | | | | | |
 | <lod 2.21<br=""><lod <lod<="" td=""></lod></lod> |
| 9/7/2015 | 5 52
460
 | 2
60

 | 269
270 | R01-H-54-8-12-2015-052-005
R07-KBL-51-11-10-2015-462- | Raisins - R01
Raisins - R07
 | Herat
Kabul
 | Medium Quality Round Green Raisin
Dried is shade and is much house-followith Khana)
Son dried Turantes Insure for the society from the
 | AF
AF | N34;18;31,6799
562:12:50:4400 | x | x
 | | x | 6.18 | × | L+ | | x | 18.27 | 235
 | <lod <lod="" <lod<="" td=""></lod> |
| | 434
 | 64

 | 271 272 | R05-KBL-51-10-10-2015-404-
R05-KBL-51-10-4-2015-365- | Raikins - R05
Raikins - R05
 | Kabul
 | north) & Abicus frame for the variets from the south)
San Strie Shomali Ruinin (an dried, black in color, has a
driven concord grant flavor for large used, often reported
San Strie Shomali Ruinin (an dried, black in color, has a
driven concord string flavor for large date, often anotated
 | AS
AS | L | EF |
 | | _ | _ | | EŦ | | _ | |
 | <lod <lod="" <lod<="" td=""></lod> |
| | 410
 |

 | 273
274 | R05-KBL-51-10-10-2015-410-
R02-KBL-51-11-10-2015-582- | Raikins - R05
Raikins - R02
 | Kabul
 | ntenar concent anne Timer bar anal kands, often anentad
San dried Shomali Rahin (sun dried, black in color, bas a
timera concent anna Timer bar anal kands, often anentad
Medium Quality Long Gmen Seedless Rakin (Dried in stade
 | AF
AF | | |
 | | | | | | | | |
 | <lod <lod<br=""><lod <lod<="" td=""></lod></lod> |
| | 469
 |

 | 275
276 | RD4-PR-51-10-28-2015-469
RD5-KBL-51-10-10-2015-422- | Raikins - RD4
Raikins - RD5
 | Parwan
Kabul
 | und is read howma blidheish Bhana)
Medium Quality Bid Raisin Juan Arled locally used raisins in
free dishas and how and share another
fam dried Shomal Raisin Juan dried, black in color, bas a
 | AF
AF | | |
 | | | | | | | | |
 | <ldd 22.70<br=""><ldd <ldd<="" td=""></ldd></ldd> |
| | 47
26
 | 0

 | 277 | R08-KBL-52-8-4-2015-047-
R06-8-52-8-3-2015-026 | Raikins - RDB
Raikins - RDB
 | Kabul
Balkh
 | Intera monutel amon Elizar bet small seads officen assorted
Enail red Raisin or currents (sus dired and stime) in dirt,
bodhumat nicibia in indus dabat and haized assoch
San dirtice Tygetter (prame for the variety from the
soch il & Abissi currents for the variety from the
soch il & Abissi currents for the variety from the
soch il & Abissi currents for the variety from the
 | 45
46 | | |
 | | | | | | | | |
 | <lod 142.95<br=""><lod <lod<="" td=""></lod></lod> |
| | 49
 | 19

 | 279 | R28-8-52-8-4-2015-049- | Raisins - RDB
 | Balkh
 | north & Abios (name for the variets from the asuft)
Forall red Raine or currents (sum dried and strengt in drie,
localizu and raine in rise dates and based secold)
defines (number the Rel Raine) must be for the current of the rest.
 | M | | |
 | | | | | | | | |
 | <100 12.40 |
| | 505
 |

 | 280
281 | R08-98-51-10-28-2015-506-
R08-8-53-8-4-2015-048- | Raikins - RD4
Raikins - RD8
 | Parwan
Balkh
 | Meetum quality waa kaalan juun ahee locaay uake halini ka
dise dahaa aa adaa daada daada
Sinaal red Raalah or currents (sun dired and stirved in dire,
Jocahu waar haniain ini ce dahaa and baked asoda)
 | AS
AS | | |
 | | | | | | | | |
 | <lod 5.24<br=""><lod 11.75<="" td=""></lod></lod> |
| | 443
 |

 | 282
283 | R05-KBL-51-11-10-2015-648-
R08-8-52-8-3-2015-028- | Raisins - RDS
Raisins - RDB
 | Kabul
Balkh
 | Inculiences minimis in circ dishea and baked encohi
San dried Showari Salani (pun dried shake): no long, ina a
stopar concerd anon fisser bat small ander, often reaported
Sanall red Salais or currents (pun dried and ander elle of dir,
pundhunant minimis inci dubas and anala sted en dub.
 | AS
AS | | |
 | | | | | | | | |
 | <lod <lod<br=""><lod 0.44<="" td=""></lod></lod> |
| | 43
 | 13
58

 | 284 | R05-8-53-6-3-2014-043-
R05-KBL-51-10-4-2015-358- | Raikins - RDB
Raikins - RDS
 | Balkh
Kabul
 | Sanaar nee kaaaan oo currenta juud enee a nei storne ai na intr.
Docalin-used hulinisii ni rice dikhea and bakke moodul
Sun dried Shormali Raksin juun dried, black in color, has a
 | AS
AS | | |
 | | | | | | | | |
 | <lod <lod<br=""><lod <lod<="" td=""></lod></lod> |
| | 52
 |

 | 286 | R08-8-53-8-4-2015-052-
R06-KBL-55-8-29-2015-264 | Raikins - RDB
Raikins - RDB
 | Balkh
Kabul
 | Insurances and some has a small seader, offen exercised
Small red Rails or currents () used field and utrine in dirt,
be allowed rails in increa fishes and haded souch)
San dried Gaussi Rainis () and fished, black in color, (
 | AF
AF | | |
 | | | | | | | | |
 | <lod 2.75<br=""><lod 29.03<="" td=""></lod></lod> |
| | 570
470
 | 20

 | 288 | R07-KBL-51-11-10-2015-570-
R02-98-51-10-28-2015-470- | Raisins - R07
Raisins - R02
 | Kabul
 | has a strong constrol grand flavor had smill work, often
San dried Typefer (name for the warley han the
work). Is abilities from the strong has smith the
Medium Qualify Long Green Sections Shak (Sinchi In Indee)
 | AS
AS | | |
 | | | | | | | | |
 | <lod <lod<br=""><lod <lod<="" td=""></lod></lod> |
| | 481
580
 | 81

 | 290 | R02-PR-51-10-28-2015-481- | Raisins - R02
 | Parwan
Parwan
 | oot is met house. Kilonish Khaoa)
Medium Quality Long Gene Seetless Rakis (Dried is stude
and is mud house-Kilonish Khaoa)
Medium Quality Long Genes Seetless Rakis (Dried is stude
 | AF | | |
 | | | | | | | | |
 | <100 1.27 |
| | 573
 | 72

 | 292 | 802-68-51-11-10-2015-580-
802-68-51-11-10-2015-573- | Raikins - R02
Raikins - R02
 | Kabul
Kabul
 | and in mud house-Klishmish Khanal
Medium Quality Long Green Seedless Rakin [Dried in shade
and in mud house-Klishmish Khana]
 | AF
AF | | |
 | | | | | | | | |
 | <lod <lod<br=""><lod <lod<="" td=""></lod></lod> |
| | 504
566
 |

 | 293
294 | R02-98-51-10-28-2015-504
R07-KBL-51-11-10-2015-566- | Raisins - R02
Raisins - R07
 | Parwan
Kabul
 | Medium Quality Long Grees Seefless Ruisin
Divid is tracked on its most Nous-Dividential National
Sun divid I Taylefee (name for the variety from the
moth) & Adolau Imare for the variety from the sauth)
End fried Taylefee (name for the variety from the
End fried Taylefee (name for the variety from the
End fried Taylefee (name for the variety from the
 | AS
AS | | |
 | | | | | | | | |
 | <lod <lod<br=""><lod <lod<="" td=""></lod></lod> |
| 10/10/201 | 556
15 325
 |

 | 295
296 | R07-KBL-51-11-10-2015-556-
R04-KBL-51-10-3-15-325-008 | Raisins - RD7
Raisins - RD4
 | Kabul
Kabul
 | Notifie & Adolas Interne for the variety from the adults
Medium Quality Red Rakin (sun died locally used rakins in
Kabul, Mandawi
 | AS
AS | N34;30;55,3600 | × | ×
 | | x | 6.72 | × | | | × | 9.32 | 13.34
 | <lod <lod<br=""><lod <lod<="" td=""></lod></lod> |
| 9/8/2015 | 579
5 56
 |

 | 297
298 | R02-KBL-51-11-10-15-579-
R02-H-52-8-10-15-056-002 | Raisins - R02
Raisins - R02
 | Kabul
Herat
 | Medium Quality Long Green Seedless Rakis Dried in shade
and is mut house. Kitheshih Khana)
Medium Quality Long Green Seedless Rakis Dried in shade
Azimi Market, Hest
 | AS
AS | N 34;20,4,5899 | × | x
 | | x | 6.19 | x | | | x | 46-20 | < 100
 | <lod <lod<br=""><lod <lod<="" td=""></lod></lod> |
| 10/7/2015 | 417
 |

 | 299 | R03-KBL-51-10-10-15-417-
R03-KBL-51-10-14-15-369-017 | Raisins - R02
Raisins - R03
 | Kabul
Kabul
 | Admi Market, Hest
Medium Quality Long Creen Seedless Rakin Diried in shade
work in work hown Althonia Manin
Nigh Quality Bundlichkei Rakin
Nigh Quality Bundlichkei Rakin
 | AS
AS | N34(30)55(00 | × | ×
 | | × | 7.12 | × | | | × | <100 | <109
 | <lod <lod="" <lod<="" td=""></lod> |
| 9/7/2015 | ន
 | 2

 | 301 | 801-0-51-8-12-2015-053-003 | Raisins - RD1
 | Herat
 | K-bitan, Ulah Value Peter in akada and in muri housa. Kabul, Mandawi Medium Quality Round Generi Nalain Poter in muri housa, kiteknish Vitana Acimi Market, Herst Small red Raisin or currents i yan dele and stored in dirty. Menze .
 | AF | EED-10-34 3000
N34(20)2,8999
EED-10-34 5400 | x | x
 | | x | 6.98 | x | | | x | 15.01 | Trace
 | <100 <100 |
| 9/8/2015
9/8/2015 | s 20
 |

 | 302
303 | R08+H52-8-9-15-011-001
R08+H53-8-9-15-0020-002 | Raikins - R08
Raikins - R08
 | Herat
 | Iscallward minimi ni nice dishea and takked reodu)
Senall red Ralain or currents (sun dried and stirred in dirt,
Iscallward minimi ni nice dishea and takked modul)
 | AF
AF | N/Av
N34;20'27,3399
562-12-0 5400 | x |
 | x | | x 7.32
x 6.73 | × | | | x | 8.68
18.47 | < 100
 | <lod 10.43<br=""><lod <lod<="" td=""></lod></lod> |
| 9/12/2015 |
 | 27
28

 | 204
205 | R02 ++ 53 -8 -11 -15 -0067 -004
R02 ++ 52 -8 -11 -15 -0069 | Raisins - R02
Raisins - R02
 | Herat
 | Medium Quality Long Green Seedless Rakin (Dried in shade
and in mud house-Kliemith Khana)
Medium Quality Long Green Seedless Rakin (Dried in shade
and in mud house-Kliemith Khana)
 | AS
AS | N34(20)39,2599
562:11:27.6700 | × | ×
 | | x | 6.88 | × | | | × | Trace | < 100
 | <lod <lod<br=""><lod <lod<="" td=""></lod></lod> |
| 9/12/2015
9/7/2015 |
 | 19

 | 306
307 | R02-H-54-0-10-15-059-003
R02-H-54-0-11-2015-072-001 | Raisins - R02
Raisins - R02
 | Herat
 | Medium Quality Long Green Seedless Rakis (Dried in shade
and in mud house-Kilomish Khana)
Medium Quality Long Green Seedless Rakis (Dried in shade
and in mud house-Kilomish Khana)
Dilabad. Herat
 | AS
AF | N34;20;4;82999
562:11:24:5400
N34;14;43;9900 | x | x
 | | x | 7.28 | × | | | x | <100
12.18 | < 100
9.67
 | <lod <lod<br=""><lod <lod<="" td=""></lod></lod> |
| 9/12/2015 | 8
 |

 | 308
309 | R01 ++ 53 + 9 2015 008
R01 ++ 52 +- 11 - 15 - 012 - 007 | Raisins - R01
Raisins - R01
 | Herat
 | Medium Quality Round Green Ralain
Dirisci u shade and in mud house-Kolmain Khana)
Medium Quality Round Green Ralain
 | AF
AF | N34(20)44(0500 | × | x
 | | x | 7.28 | x | | | × | 5.00 | <100
 | <lod <lod="" <lod<="" td=""></lod> |
| 9/12/2015 | 15 15
 | IS

 | 310 | 802-H-52-8-12-15-015-005 | Raisins - R02
 | Herat
 | and a market water and a more a more and a more an
 | AS | N34(20)38,3600 | x | × | | x | 7.22
 | х | | | x | 5.00 | < 100 | <100 <100
 |
| 9/12/2015
9/7/2015 | 5 14
 | 14

 | 311
312 | 801-H 53-8-9-15-013-006
801-H 53-8-12-2015-014-002 | Raisins - R01
Raisins - R01
 | Herat
 | Dried is shade and in mot becau Alchmish Khanal Jada e-Hesar, Mandawi, Herat Medium Davilly Brown Geam Raise
 | AF
AF | N34(20)39,7299
E42141-22,2250
N34(20)39,6199
E42141-27,0890 | x | x
 | | x | 6.84 | x | | | x
x | 5.52
24.79 | < 100
 | <lod <lod<br=""><lod <lod<="" td=""></lod></lod> |
| 10/7/2015 | 491
 | 44

 | 313
314 | 806-98-51-10-28-2015-491-
803-KBL-51-6-10-15-346-022 | Raisins - R06
Raisins - R03
 | Parwan
Kabul
 | Printe hardes well in much houses all blockship blockst. John of Heiser, Mandawi, Herst
San dheid Ghansi Rainin Jourd deid, block in color;
Inse a strinor proceediment Block hardes and lander aPhen
High Quilly Shundurdhost Rainin Schler Hardes and Hardes Hardes (Hendrechter Hardes)
Gallen-stieft villes Grief in shade and thouses-
Gallen-stieft villes Grief in shade and thouses-
 | AS
AS | N34;30;25,0200
669:4:30:35:300 | x | ×
 | | x | 6.72 | x | F | [| × | <100 | 5.19
 | <lod <lod<br=""><lod <lod<="" td=""></lod></lod> |
| _ | 352
362
 | 42

 | | R05-KRI-51-10-6-2015-352
R06-KN-53-9-9-2015-242- | Raisins - R05
Raisins - R08
 | Kabul
Kandahar
 | Kolden-Hah Value Dried in shade and in mut house-
Ban dried Shomali Ruinin (uu dried, bluck in calos), hus a
drane record and anno Hauri Al un deal, often resorted
Small nee Ruinin or currents (uu dried and stirred in drit,
scallurus af nisis in for drikus and blacket ancohi
Bandin et Ruinin for drikus and blacket ancohi
 | AF
AF | \pm | L-F |
 | | _+ | | E- | LŦ | | | |
 | <lod <lod<br=""><lod 11.89<="" td=""></lod></lod> |
| | 495
 |

 | 317
318 | R06-PR-51-10-28-2015-496-
R06-PR-51-10-28-2015-479- | Raikins - R06
Raikins - R06
 | Parwan
Parwan
 | In carry used reasons in one defines and taxets exection
Sound Herd Character Reason (Survey Carry Context)
has a stronger conserved reason (France) based and the second of the
Sound Herd Character Reason (France) based in colore.
 | AF
AF | | + | -
 | + | - | | | | _ | | |
 | <lod <lod="" <lod<="" td=""></lod> |
| | 518
 | 28

 | 319
320 | R04-98-51-10-29-2015-518-
R08-8-52-8-4-2015-053- | Raisins - R04
Raisins - R08
 | Parwan
Balkh
 | has a stream concerd areas flavor but small seeds, often
Medium Quality the fixation (sun their locally used nation in
fixed chinas and busiked needs)
femall med hashin or currents (sun dhied and strengt in dirt,
incrulinuant chinami inclus chinas with busied needs)
 | AS
AS | - | \square |
 | | - | | | | | | |
 | <lod 12.68<br=""><lod 1.89<="" td=""></lod></lod> |
| 10/10/17 | 54
265
15 382
 | 65

 | 321
322 | 806-881-55-8-29-2015-265-
805-881-55-8-29-2015-265-
803-881-51-4-10-15-382-021 | Raisins - R08
Raisins - R06
Raisins - R03
 | Balkh
Kabul
Kabul
 | localivuand rahim in rise dishm and baked asods)
San dired Ghasa Rahim (pun dired, black in color,
Mara a tensor second second fiscan disacch at seall useds often
High Quality Dundarithani Rahim
 | A5
A5 | N34;30;54,2799 | x | x
 | | , | 7.35 | v | | | x | 5.00 | < 100
 | <100 1.89
<100 1.26
<100 <100 |
| 10/10/2011
9/13/2015 | 15 91
 | 21

 | 323 | 802-0-52-8-11-15-091-008 | Raisins - R02
 | Herat
 | ngin quality suizueurus naun Kabul, Mandawi Kabul, Mandawi Mabul, Mandawi Medium Quality Long Green Seedless Rakin (Dried in shade
wordin much boxum Litherholt Nauna) Medium Quality (Medium Quality Medium Quality Medium Quality Medium) (Medium (Mediu
 | AS | 669-10-25-0500
N34(20,29,2599
662-11-27-6300 | x | × | | x | 7.35
 | x | | | x | 5.00 | < 100 | <100 <100
 |
| 10/7/2015 |
 | 23

 | 324
325 | R04-PR-51-10-28-2015-510-
R03-KBL-51-10-3-15-333-012 | Raisins - R04
Raisins - R03
 | Parwan
Kabul
 | Medium Quality Fed Rainin juun dried locally used naisins in
dra drihan wet haised annohit
MgR Quality Rundlutchart Rainin
Koldun, Mandawi
Koldun, Mandawi
 | AS
AS | N 34:30:55,8099
649-10-35 0300 | × | ×
 | | x | 7.71 | x | | | x | <100 | <100
 | <lod 2139.30<br=""><lod <lod<="" td=""></lod></lod> |
| 10/10/201 | 519
15 381
 | 81

 | 326
327 | R04-98-51-10-29-2015-519-
R04-KBL-51-4-10-15-381-010 | Raikins - RD4
Raikins - RD4
 | Parwan
Kabul
 | Medium Quality Red Rakin (sun dried locally uned rakins in
drin drihan and heiserd anochi
Medium Quality Red Rakin (sun dried locally uned rakins in
drin drihan and heiserd anochi
 | AF
AF | N34(30)54,2200
569:10:35:1099 | x | ×
 | | x | 6.65 | x | LŦ | | × | 8.21 | 12.54
 | <100 2.21
<100 <100 |
| 10/7/2015 | 565
15 360
 |

 | 328
329 | R02-KBL-51-11-10-2015-565-
R03-KBL-51-10-4-2015-360-019 | Raisins - R02
Raisins - R03
 | Kabul
Kabul
 | Inte sinue and taked record
Medium Quilly Cong Green Seedless Rabin Dried in shade
and in matchouse kilomish Rusual
High Quilly Switchard Rabin
Biolden shith Value Dried in shade and in mult house.
Roders shith Value Dried in shade and in mult house.
 | AS
AS | N 34;30;55,2899
669:10:33 880** | x | ×
 | | x | 7.07 | x | LT | | × | <100 | < 100
 | <lod <lod="" <lod<="" td=""></lod> |
| 10/13/201 | 429
 | 25
29

 | 220
221 | R05-KBL-51-4-10-2015-375-024
R05-KBL-51-10-10-2015-429- | Raikins - R05
Raikins - R05
 | Kabul
Kabul
 | Kalden Steht Valan Cirkel in badet and in mark house. Sun drived Shomal Rakin (sun drived Shock in color, hus a Kabud, Mandawi Son drived Shomal Rakin (sun drived, black in color, hus a Kabud, Mandawi Son drived Shomal Rakin (sun drived, black in color, hus a
 | AF
AF | Lat: 34,30548299
Lone: 69,103464200 | x | ×
 | | x | 6.44 | x | | | x | <100 | 45.31
 | <lod <lod<br=""><lod 36.79<="" td=""></lod></lod> |
| 10/7/~* | 429
497
15 321
 |

 | 222
222 | RD4-PR-51-10-28-2015-429-
RD4-PR-51-10-28-2015-497-
RD3-KBL-51-10-3-15-321-010 | Raikins - RD4
Raikins - RD4
 | Parwan
Kabul
 | Interner concerd ansee flavor but small seeds, often essented
Medium Quality fred Rakin Juan Attect to cally used rakins in
free drives and basinked needel
Nigh Quality Shurduchhan Rakin
Perfore Little him Poter in selen and in much heren
 | л
л
л | N34;30;55,6499 | x | ×
 | | , | 2.34 | v | | | x | <100 | < 100
 | <100 36.79
<100 1.26
<100 <100 |
| 10/7/2015
9/13/2015
30/6/2015 | 15 224
 | 24

 | 234 | R01-KN-52-9-9-15-0224-009 | Raisins - RD1
 | Kandahar
 | Medium Quality Round Green Rakin
Medium Quality Round Green Rakin
Disisti untuks and in mut becara Michaelsh Phanat
Haladi un Quality Round Green Rakin
 | AF | 160-10, 26,0400
N 21;34;25,27994
E55:48:50,000
N 34;30;54,6100 | x | ×
 | | x | 6.46 | × | | | x | 14.97 | < 100
 | <100 <100 |
| 10/6/2015 | 474
 | 74

 | 225 | R01-KBL-51-4-10-15-290-018
R06-98-51-10-28-2015-474- | Raikins - R01
Raikins - R06
 | Kabul
Parwan
 | Triade is what a not in much house. Michaelsh Yhanasi
San dried Ghanni Railin Juan dried, black in color,
Nan a shrear concred amate fiberic hud small andre often
 | AF
AF | EED-10-34 0500 | x | ×
 | | × | 6.28 | x | | | x | <100 | <100
 | <lod <lod<br=""><lod 1.00<="" td=""></lod></lod> |
| 10/6/2015
10/13/2015 | 15 454
 | 54

 | 228 | R01-KBL-51-4-10-15-377-017
R02-KBL-51-10-11-2015-454-013 | Raisins - R01
Raisins - R02
 | Kabul
Kabul
 | Medium Quality Round Green Railon
Driviel in whole word in word in word horses Minimal Manual
Medium Quality Cong Greens Geolesis Railon (Dried in shade
word in word horses Kithenish Khans)
 | AF
AF | N34(30)54,4600
668-10-35 1400
N34(30)55,2599
668-10-35 2500 | x | x
 | | x
x | 6.34
6.66 | x
x | F | | x
x | <lod
<lod< td=""><td>< 100</td><td><lod <lod<br=""><lod <lod<="" td=""></lod></lod></td></lod<></lod
 | < 100
 | <lod <lod<br=""><lod <lod<="" td=""></lod></lod> |
| 9/13/2015 | 433
 | 23

 | 340
341 | R01-KN-51-9-9-15-0225-0012
R05-KBL-51-10-10-2015-433 | Raikins - R01
Raikins - R05
 | Kandahar
Kabul
 | Anne and Ann
 | AS
AS | N 21:34:25:2400
E65:48:50:10909 | x | × | | x | 7.3
 | x | LT | | x | 14.03 | < 100 | <lod <lod="" <lod<="" td=""></lod>
 |
| 10/10/201 | 15 342
 | 42

 | 342 | R04-KBL-51-10-6-15-342-006
R01-KBL-51-6-10-15-380-016 | Raikins - R04
Raikins - R04
 | Kabul
 | Medium Quality Red Raisin (sun dried locally used raisins in
rice dishes and baked recodul
 | AF
AF | N34;30;25,0200
669:4:30:35,3000
N34;30;54,4600
669:10:35,1400 | x | x
 | | x | 6.85 | x | \square | | x
x | 7.82
8.93 | <100
 | <lod 14.05<="" td=""></lod> |
| 10/10/201 | 475
 | 75

 | 342 | R04-98-51-10-28-2015-475-
R04-98-51-10-28-2015-475-
R08-KN-52-9-8-15-227-004 | Raisins - R04
Raisins - R04
Raisins - R08
 | Parwan
Kandahar
 | Orea in utage and in the focus outputs status
Medium Quality Ref Raini (suc dired locally used raisins in
rice dishes and baked seeds)
 | A
A
A | 569:10:35:1400
N21:34(27,660 | x | -
 | 1 | | 636 | x | | | v | 13.66 | 25.68
 | <lod 2.93746233<="" td=""></lod> |
| | 370
 | 20

 | 346 | R09-KBL-51-10-6-2015-370- | Raisins - R09
 | Kabul
 | Other OR Mixed Ralain
 | AF | 165-48-47.190
N34-30.54 46*** | | ×
 | | | | | | | x | |
 | <100 <100 |
| 10/10/2011
10/7/2015 | 15 201
 | 01

 | 347
348 | 804 KBL-51-10-5-15-395-012
803-KN-51-9-8-15-2015-005 | Raisins - RD4
Raisins - RD3
 | Kabul
Kandahar
 | Medium Quality feel Falain juun dried locally used raisins in
Kabud, Shahr 4-N aw
Kabud, Shahr 4-N aw
High Quality Shundurshani Raisin
Polities Like Valain Polisi in Indee Socially used raisins in
Medium Quality Re Raisin juun dired Socially used raisins in
Dired Montenaul
 | AF
AF | N34/30/54/4600
F69-10/34/5299
N31/34/27/729
F65-49-47/829
N34/30/54/4600 | x | x
 | | x | 6.75
7.34 | x | | | x
x | 5.90
<lod< td=""><td>16.38
14.19</td><td><lod <lod<br=""><lod <lod<="" td=""></lod></lod></td></lod<> | 16.38
14.19
 | <lod <lod<br=""><lod <lod<="" td=""></lod></lod> |
| 10/10/2011
10/7/2015 | 15 234
 | 24

 | 250
251 | R04-KBL-51-4-10-15-386-011
R03-KBL-51-10-3-15-334-008 | Raikins - RD4
Raikins - RD3
 | Kabul
Kabul
 | idea dahas and haland anodal
High Quality Dendukthani Ralain
Kabul, Mandawi
 | AF
AF | 260-10-35 1000 | x
x | x
 | $\pm \neg$ | x
x | 6.92
6.85 | x
x | L-T | | x
x | 7.92
<lod< td=""><td>20.01
111.71</td><td><lod 0.67<br=""><lod <lod<="" td=""></lod></lod></td></lod<> | 20.01
111.71
 | <lod 0.67<br=""><lod <lod<="" td=""></lod></lod> |
| 10/6/2015 | 15 362
 |

 | 352
354 | R01 KBL-51-6-10-15-362-023
R05 KBL-51-10-6-2015-388 | Raisins - R01
Raisins - R05
 | Kabul
 | Medium Quality Round Green Raisin
Driant in shada and in muri herana Alabashi Wannal
San dried Shamali Raisin Isun dried, black in color, has a
 | AS
AS | N34(30)55,5800
660-10-33 8099 | × | ×
 | | x | 7.24 | × | | | x | <100 | < 100
 | <lod <lod<br=""><lod <lod<="" td=""></lod></lod> |
| 10/7/2015
10/13/2015 | 15 322
 | 22

 | 255
256 | R03-KBL-51-03-10-15-322-009
R04-KBL-51-10-11-15-658-007 | Raikins - RDJ
Raikins - RD4
 | Kabul
Kabul
 | stoper concord ansen flavor but small sends, often exported High Quality Shunduchen Rasin Kabul - Mandawi Kabul - Mandawi Medium Quality Red Rasin Juan dried locally used rakins in Kabul - Mandawi
 | AF
AF | N34(30)55,5399
569-10-35-7200
N34(30)55,2899 | x | x
 | | x | 6.24 | × | | | x
x | 8.17
<lod< td=""><td><100</td><td><lod <lod<br=""><lod <lod<="" td=""></lod></lod></td></lod<> | <100
 | <lod <lod<br=""><lod <lod<="" td=""></lod></lod> |
| 10/7/2015 |
 | 27

 | 257
257 | RD4-KBL-51-10-13-2015-327-001
RD1-KBL-51-10-3-2015-328- | Raikins - RD4
Raikins - RD1
 | Kabul
Kabul
 | nee ontwis indicated recently
Medium Qualify See Fability fan Ender
See dithes and baked recently
Medium Qualify Round Green Rahim
 | AF
AF | E69-10-35.4700
N34;30;55,8999
E69-10-35.6799 | x | ×
 | | x | 6.29 | × | | | × | <100 | < 100
 | |
| | 426
 | 26

 | 258
260 | RD5-KBL-51-10-10-2015-426-
RD5-KBL-51-10-10-2015-426-
RD5-KBL-51-10-10-2015-420- | Raisins - RDS
 | Kabul
 | Dried in shade and in mud house-Kahmah Khana)
San dried Shomal Rainin (un dried, black in color, has a
stroare concost anaoe flavor bat small seeds, often excerted
San dried Shomal Rainin (un dried, black in color, has a
 | 77
14
14 | | |
 | | | | | | | | |
 | <100/<100 <100/<10
<100 <100 <100 |
| 10/7/2015 | 15 361
 | 61

 | 361 | R03-KBL-51-10-3-15-361-015 | Raisins - R05
Raisins - R03
 | Kabul
Kabul
 | strone cencerd intee fliver but avail useds often exzerted
Nigh Quality Strondowin Russin
Forders site Value Fried in stade and in mod house.
Kabul, Mandawi
 | AS | N34(30)55,5800
009-10-33-8999 | × | ×
 | | x | 6.66 | × | | | × | <lod< td=""><td>< 100</td><td><100 <100</td></lod<> | < 100
 | <100 <100 |
| | 465
 |

 | 262
263 | R04-98-51-10-28-2015-465-
R02-KBL-51-10-10-2015-401- | Raisins - RD4
Raisins - RD2
 | Parwan
Kabul
 | Medium Quality Rod Rasian juun dired locally used naisite in
fice dishara such baland secold
Medium Quality Long Green Seedless Rabia (Dried in shade
work on work house). Nahmah Nahmah
 | A5
A5 | | |
 | | | | | | | | |
 | <lod 11.38<br=""><lod <lod<="" td=""></lod></lod> |
| | 461
 |

 | 264
265 | R07-KBL-51-11-10-2015-461-
R05-KN-52-9-10-15-243- | Raisins - R07
Raisins - R05
 | Kabul
Kandahar
 | and is much house. All and hit has all
Sun dried Tayelie (name for the unity from the
evolution of the state of the units of the state)
Sand dried Shormali Rabin (sun dried, black in color, has a
driven or ensured more fill such as all south of the state)
 | AS
AS | | | _
 | | | | | | | | |
 | <lod <lod<br=""><lod 12443<="" td=""></lod></lod> |
| | 402 262
 | 02

 | 266 | RD5-KR-52-9-10-15-243-
RD5-KR-51-10-10-2015-402-
RD5-KR-34-9-25-15-242-002 | Raisins - RDS
Raisins - RDS
Raisins - RDS
 | Kabul
Kabul
 | etenar concent annue liner bet anali sande often annotad
San dried Shemali Rakin (au dried, black in color, hus a
minner concent annue liner bet ar anali sande often annotad
San dried Shemali Rakin (au dried, black in color, hus a
 | 45
45
45 | | |
 | 1 | | | | | | | |
 | <100 1344
<100 0.58
<100 1.86 |
| 9/13/2015 | 287
 | 87

 | 367
368
369 | R09-KBL-51-10-6-2015-387- | Raisins - R09
 | Kabul
 | stone concest areas flavor but small seeds, often experted
Other OR Mixed Raisin
 | AF
AF | N21;34;25,389 | | x
 | | | | × | | | × | |
 | <100 <100 |
| 9/13/2015 |
 |

 | 269 | RD1-KN-52-9-9-15-0223-0010
RD5-KBL-51-4-10-2015-389-025 | Raisins - R01
Raisins - R05
 | Kandahar
Kabul
 | Metauru guary securit naan
Dinisi la valade and in mud house kolumiah Klama)
Dinisi di valade and in mud house kolumiah Klama)
San dindi shamal Rabia (juun dind, black in color, tas a
itoar concod annon flavor but small seeds, offen reasorted
Metauru guary Long Green Seeden, Rabia (Dinis in India
 | AS | N 82,342,25,889
665-48-50,140
N 34,30,54,6100
669-10-34,9599 | x | ×
 | | x | 6.32
6.48 | × | | | x | 15.90
6.59 | 8.01
<100
 | <lod <lod<br=""><lod <lod<="" td=""></lod></lod> |
| 10/7/2015 | 411
 |

 | 371
372 | R02-KBL-51-10-10-2015-411-
R03-KN-51-9-8-15-216-002 | Raikins - R02
Raikins - R03
 | Kabul
Kandahar
 | and in mud house-Kishmish Khana)
High Quality Shundukhani Raisin
Santon Muduhan Sanghesar dist. Kandahar
 | AS
AS | N 31, 34, 25, 5700 | × | x
 | | x | 6.85 | x | | | x | 5.97 | 148.94
 | <lod <lod<br=""><lod <lod<="" td=""></lod></lod> |
| 10/13/201 | 490
 |

 | 272
274 | R04-PR-51-10-28-2015-490-
R06-KBL-51-10-10-15-429-022 | Raisins - RD4
Raisins - RD6
 | Parwan
Kabul
 | Maderum calari ya Ka dabin ju na dela locally used nalini in
dra dhau vol halvot moduli
San dried Ghasni Ralain (sun dried, block in color,
has a strong concert grane Binovich e unali sande "Atan. Kabul, Mandawil
 | AS
AS | N 34(30)53,9199 | × | x
 | | x | 6.69 | × | | | × | 5.91 | < 100
 | <lod 88.59<br=""><lod <lod<="" td=""></lod></lod> |
| | 405
 |

 | 375
376 | R05-KBL-51-10-10-2015-405-
R09-KN-52-9-10-2015-241- | Raikins - RDS
Raikins - RDB
 | Kabul
Kandahar
 | has a strong reported range financiella snaft, often
San dried Shomali Rakin (sun dried, black in color, has a
strong reported range financie kar and standar ding particular
Small red Rakin or currents (sun dried and stirred in dirt,
 | AS
AS | 100-10-20 2000 | |
 | | | | | | | | |
 | <00/400 <100/<10
<100 4.02 |
| | 480
 | eo ce

 | 377 | R04-PR-51-10-28-2015-480-
R08-R-52-8-4-2015-051- | Raisins - RD4
 | Parwan
 | In-allument minister in vice minister and halond anouth
Medium Quality Red Rainin (sum dhed locally used rainins in
vice riskes work halond enouth)
Famil red Rainin or currents (sum dhed and stirved in dirt,
 | AF | | |
 | | | | | | | | |
 | |
| 10/7/2015 | 15 284
 | 84

 | 279 | R03-KBL-51-10-6-2015-384-020 | Raikins - RDB
Raikins - RDD
 | Balkh
Kabul
 | In railward militer in rine diaher and heixed models
High Quality Shundurkhani Railan
Golden High Valae Dried in shade and in mud house-
 | AF
AF | N34(30)54(4600
669:10:34(7500 | × | x
 | | | 6.29 | | | | x | <100 |
 | <100 2.57 |
| 10/6/2015 |
 |

 | | R04-PR-51-10-28-2015-529-
R01-KBL-51-4-10-15-351-021 |
 |
 |
 | AF
AF | | |
 | | X | | × | | | | <lod< td=""><td>< 100</td><td><lod 102.80<br=""><lod <lod<="" td=""></lod></lod></td></lod<> | < 100
 | <lod 102.80<br=""><lod <lod<="" td=""></lod></lod> |
| 10/10/201 |
 |

 | 380
281 | | Raikins - RD4
Raikins - RD1
 | Parwan
Kabul
 | Medium Quality Red Rakin (sun dried locally used nakins in
dice drives and bained secold)
Medium Quality Round Green Rakin
Dried in taked and is mud house-Kühmöh Khanal
Rahul, Mandawi
 | | N34;30;55,8999
669:10:33,4200 | × | x
 | | x | 6.45 | x | | | × | | < 100
 | <lod 102.80<br=""><lod <lod<br=""><lod 4.38<br=""><lod <lod<="" td=""></lod></lod></lod></lod> |
| 10/6/2015
10/10/2015 |
 | 91
24

 | 381
382
383 | R04-KBL-51-10-4-15-291-009
R03-KBL-51-10-3-15-324-007 | Raikins - R01
Raikins - R04
Raikins - R03
 | Kabul
Kabul
Kabul
 | for al dhina and basics proceds
Medium Quality Round Gereen Rasin
Minist In Statute and In use Insues Scherbis Manual
Medium Cashing You Sharoling In Medium Cashina In
Rep Quality Roundwethani Rasin
Medium Cashing You Sharoling In Statute Cashina In
Rep Quality Roundwethani Rasin
Kabul - Mandawi
Kabul - Mandawi
 | AF
AF | E69-10-33.4200
N34(30)54(5000
E69-10-34.8599
N34(30)55,3300
E69-10-35.6799 | x x x x x x x x x x x x x x x x x x x |
x
x
x | | x
x
x
x | 6.98 | | | | x
x
x | 7.68 | <100
12.21
<100
 | <1.00 |
| _ | 15 368
 | 91
24
68

 | 381
382 | 804-48-51-10-4-15-391-009
803-48-51-10-3-15-224-007
801-48-51-410-15-368-019
805-48-51-10-5-15-399-003 | Raisins - RD1
Raisins - RD4
 | Kabul
Kabul
Kabul
Kabul
 | an dia mandra dan dia
 | N
N
N
N | E59-10-33-4200
N34-30;54,5000
E59-10-34,8599
N34-30;55,3300
E59-10-35,57300
E59-10-34,7599
N34:20;54,9700
E59-10-34,7599
N34:20;54,9500
E59-10-34,7599
N34:20;54,4500 | × | x
 | | x | 6.98 | × | | | x | 7.68 | <100
 | 400 102.00 400 400 400 400 400 400 400 400 400 400 400 400 400 400 400 400 400 400 400 400 400 400 |
| 10/7/2015 | 15 364
15 299
 | 91
24
68
99
29

 | 281
282
283
284 | R04 KBL 52-10-6-15-281-009
R03-KBL 52-10-3-15-226-007
R01-KBL 52-6-10-15-368-019 | Raikins - RD1
Raikins - RD4
Raikins - RD3
Raikins - RD1
 | Kabul
Kabul
Kabul
Kabul
Kabul
 | Name and a set of the
 | AS
AS
AS | EEE-10.32.4200
N34.20;54,500
EEE-10.34.8599
N34.20;55,3200
EEE-10.35.6799
N34.20;54,9709
EEE-10.34.7299
N34.20;54,4000 | x
x
x | x
x
x | | x
x
x | 6.98
6.25
6.80
 | x
x
x
x | | | x
x
x | 7.68
17.14
Trace | <100
1221
<100
428 | <1.00
 |
| 10/7/2015 | 15 368
15 399
15 229
50
511
 | 91
24
68
99
29
20
11

 | 281
282
283
284
285
285
286
287
288 | 804 482 43 10 4 15 381 009
803 482 43 10 3 5 38 009
803 482 43 40 15 380 019
804 482 43 40 15 380 019
805 482 43 10 5 5 5 399 003
805 482 43 10 3 5 15 399 014
805 451 45 42 315 005
804 98 51 40 28 303 511 | Rahkins - RD1
Rahkins - RD4
Rahkins - RD3
Rahkins - RD1
Rahkins - RD6
Rahkins - RD6
Rahkins - RD6
Rahkins - RD8
Rahkins - RD8
 | Kabul
Kabul
Kabul
Kabul
Kabul
Balih
Parwan
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Date of File Seq. N	Number	Random Number	Sample ID	Commodity	Province	Type of Sample	Sampling Location	Country	GPS Location	Individual Sample Log	Sampling Control Log	Elisa Co		pH Afebanistan					Romer	UNL Romer	UNL Neoeen	Austria	Aust
										Yes No	Yes No	Yes	No	• • • •	Aflatoxin	DON	T-2	Ochratoxin	Aflatoxin ppb	Aflatoxin ppb	Aflatoxin ppb	Aflatoxin ppb	Ochrato
/14/2015 8	85	551	WN01-S-S1-10-31-15-085-005	Walnut - WN01	Samangan	Zard Walnuts (yellow kernels)	Bala bazar, Samangan	AF	N36;15771 £68:01259	x	x	х		6.88	х				24.62	< LOD	< LOD	< LOD	< 10
5	54	552	WN01-S-S1-10-30-2015-054-	Walnut - WN01	Samangan	Zard Walnuts (yellow kernels)		AF												Trace	Trace	< LOD	<1
4	488	553	WN02-PR-51-10-28-2015-488-	Walnut - WN02	Panwan	Mazaari Walnuts (variety from Mazar with unique flavor)		AF												< LOD	Trace	< LOD	<
11/14/2015	70	554	WN01-S-S2-10-31-15-070-006	Walnut - WN01	Samangan	Zard Walnuts (yellow kernels)	Bala Bazar, Samangan	AF	N36;15747 E68-01296	x	х	х		6.91	х				22.35	< LOD	< LOD	< LOD	<
6	65	555	WN06-S-S1-10-30-2015-065-	Walnut - WN06	Samangan	Other varieties of Walnuts		AF												8.00	< LOD	< LOD	<
10/17/2015 4	442	556	WN05-K8L-S1-10-11-15-442-003	Walnut - WN05	Kabul	Kaghazi Walnuts (paper shelled)	Kabul, Mandawi	AF	N34;30;53,8899	x	x	x		7.68	x				<lod< td=""><td>< LOD</td><td>< LOD</td><td>< LOD</td><td></td></lod<>	< LOD	< LOD	< LOD	
10/27/2015	77	557	WN03-8-54-8-4-15-077-001	Walnut - WN03	Balkh	Takhari Walnuts(variety from Takhar province with	Dry fruit market, Mazar	AF	E69:10:35.2200 N36;42;4,0599	×	×	×		7.65	×				Trace	< LOD	< LOD	<10D	
10/27/2015	31	558	WN02.8.51.8.3.15.031.001	Walnut - WN02	Balkh	unique flavor) Mazaari Walnuts (variety from Mazar with unique	Shah Muhammad dry fruit market, Mazar	AF	E67;6;50,0700 N36;42;5,04000	×	x	x		6.67	×				Trace				-
	560	559	WN01-K8L-S1-11-10-2015-560-	Walnut - WN02	Kabul	flavor) Zard Walnuts (yellow kernels)	shan Muhammad dry Irbit market, Mazar	AF	£67:6:48.01999		^	~		6.07	^				11828		t'		
						Zard Walnuts (yeable kernets)																	-
	82	560	WN06-H-S4-8-12-2015-0082-	Walnut - WN06	Herat			AF	N34;30;54,9299												ļ'		
	424	561	WN04-K8L-S1-10-10-15-424-001	Walnut - WN04	Kabul	Korak Walnuts (opening in shell)	Kabul, Mandawi	AF	E69;10;35,3999	x	x	х		6.86	х				7.68		ļ'		
5	561	561	WN03-KBL-S1-11-10-2015-561-	Walnut - WN03	Kabul	Takhari Walnuts(variety from Takhar province with unique flavor)		AF															
10/27/2015 3	376	563	WN06-K8L-S1-10-4-15-376-011	Walnut - WN06	Kabul	Other varieties of Walnuts	Kabul, Mandawi	AF	N34;30;54,5700 E69:10:35.0700	x	х	х		7.28	х				5.34				
5	577	564	WN02-KBL-S1-11-10-2015-577-	Walnut - WN02	Kabul	Mazaari Walnuts (variety from Mazar with unique flawer)		AF														1	
5	91	565	WN01-S-S2-10-31-2015-091-	Walnut - WN01	Samangan	Zard Walnuts (yellow kernels)		AF														i	
10/5/2015 3	302	566	WN06-KBL-S1-3-10-15-302-002	Walnut - WN06	Kabul	Other varieties of Walnuts	Kabul - Mandawi	AF	N34;33;25,1999	x	x	х		6.70	x				42.07		ا		-
	301	567	WN06-KBL-S1-10-3-2015-301-010	Walnut - WN06	Kabul	Other varieties of Walnuts	Kabul - Mandawi	AF	E69:6:52.2999 N34;33;25,1999	x	x	х		7.05	x				< LOD				+
	300	568	WN06-KBL-S1-10-3-15-300-009	Walnut - WN06	Kabul	Other varieties of Walnuts	Kabul - Mandawi	AF	E69:6:52.2999 N34;33;25,199	x	x	x		6.95	x				<100				<u>+</u>
	32	569	WN06-8-53-8-3-15-032-007	Walnut - WN05	Ralkh	Other varieties of Walnuts	Safi dry fruit market. Mazar	AF	E69:6:52.9999 N36;42;5,0400	×	×	x		6.82	×				<100				+
							san ory fruit market, Mazar		E67;6;48,01999	x	x	х		6.8Z	x				<100		']	+
	503	570	WN04-PR-S1-10-28-2015-503-	Walnut - WN04	Parwan	Korak Walnuts (opening in shell)		AF	N34:30:55.899												'		
	359	571	WN04-K8L-S1-10-4-15-359-001	Walnut - WN04	Kabul	Korak Walnuts (opening in shell)	Kabul, Mandawi	AF	E69:10:33.850	x	x	х		6.75	х				10.55		ļ'		
10/17/2015 4	431	572	WN01-K8L-S1-10-10-15-431-004	Walnut - WN01	Kabul	Zard Walnuts (yellow kernels)	Kabul, Mandawi	AF	N34;30;53,8200 E69:13:35.3200	x	×	х		7.10	х				< LOD				
6	66	573	WN06-S-S1-10-30-2015-066-	Walnut - WN06	Samangan	Other varieties of Walnuts		AF													'	. 1	
10/17/2015 4	400	574	WN06-KBL-S1-10-10-15-400-006	Walnut - WN06	Kabul	Other varieties of Walnuts	Kabul, Mandawi	AF	N34;30;55,8300 E69-10-35 8999	x	х	х		7.25	х				5.50			1	
10/17/2015	57	575	WN05-H-S3-8-10-15-057-004	Walnut - WN05	Herat	Kaghazi Walnuts (paper shelled)	Azimi market, Herat	AF	N34;20;4,300 E62:11:24.6099	x	×	х		7.20	х				5.90			i	
4	471	576	WN05-PR-S1-10-28-2015-471-	Walnut - WN05	Parwan	Kaghazi Walnuts (paper shelled)		AF	102:11:24:00/9														
10/22/2015	58	577	WN05.H.St.R.10.15.058.012	Walnut - WN06	Herat	Other varieties of Walnuts	Azimi market, Herat	AF	N34;20;4,3399	×	×	×		7.00	×				5.34				
	430	578	WN06-K8L-S1-10-10-15-430-005	Walnut - WN06	Kabul	Other varieties of Walnuts	Kabul, Mandawi	AF	E62;11;24,7900 N34;30;35,6699	×	x	x		6.90	×				<100	7.83	< LOD	<100	
	407	579	WN05-K8L-S1-10-10-15-407-09	Walnut - WN05	Kabul	Kaghazi Wahuts (paper shelled)	Kabul, Mandawi	AF	E69:13:35.0700 N34;30;54,500	×	x	x		7.01	×				< 100	<100	< LOD	< LOD < LOD	
	-								E69:10:35.0400 N34;30;54,1000		×	x			×							< 100	-
	413	580	WN06-KBL-S1-10-10-15-413-008	Walnut - WN06	Kabul	Other varieties of Walnuts	Kabul, Mandawi	AF	E69:10:34.7799 N36;42;4,0599	×				7.00					6.34	Trace	< LOD		
	73	581	WN05-B-S1-8-4-15-073-007	Walnut - WN05	Balkh	Kaghazi Walnuts (paper shelled)	Dry fruit market, Mazar	AF	E67;6;50,0700	x	x	х		6.71	х				< LOD	11.65	< LOD	< LOD	
10/5/2015 4	41	582	WN06-8-54-8-14-15-041-003	Walnut - WN06	Balkh	Other varieties of Walnuts	Safi dry fruit market, Mazar	AF	N36;42;45,4999 E67:6:22.7799	x	x	х		6.85	х				41.59	< LOD	< LOD	< LOD	
10/5/2015	22	583	WN06-H-S5-8-4-15-022-001	Walnut - WN06	Herat	Other varieties of Walnuts	Blood Bank St., Herat	AF	N34;20;38,4299 E62:11:26.4100	x	х	х		6.57	х				39.64	< LOD	< LOD	< LOD	
40/17/2015	459	584	WN01-KBL-S1-10-11-15-459-002	Walnut - WN01	Kabul	Zard Walnuts (yellow kernels)	Kabul, Mandawi	AF	N34;30;55,2200 E69:10:35.2200	×	×	×		7.29	х				< LOD	5.30	< LOD	< LOD	
10/5/2015 3	326	585	WN05-K8L-S1-10-3-15-326-001	Walnut - WN05	Kabul	Kaghazi Walnuts (paper shelled)	Kabul - Mandawi	AF	N34;30;56,0500 E69:10:36.0400	x	x	х		7.70	х				36.67	<100	< LOD	< LOD	
4	467	585	WN04-PR-51-10-28-2015-467-	Walnut - WN04	Parwan	Korak Walnuts (opening in shell)		AF	247.10.30.0404											11.97	< LOD	< LOD	
10/5/2015 4	46	587	WN06-8-53-8-3-15-046-004	Walnut - WN06	Balkh	Other varieties of Walnuts	Dry fruit market of Md. Ibrahim, Mazar	AF	N36;42;45,929	x	x	х		6.42	x				40.50	Trace	< LOD	<lod< td=""><td></td></lod<>	
	144	588	WN04-S-S2-11-1-2015-144-	Walnut - WN04	Samangan	Korak Walnuts (opening in shell)		AF	£67:6·23.4999											15.88	< LOD	< LOD	
	408	589	WN05-K8L-S1-10-10-15-408-008	Walnut - WN05	Kabul	Kashazi Walnuts (paper shelled)	Kahul Mandawi	AF	N34;30;54,5700	×	x	×		6.78	×				<100	Trace	< 100	< 100	
									E69:10:34.0000 N34:33:25.1999														-
	303	590	WN06-K8L-S1-10-3-15-303-014	Walnut - WN06	Kabul	Other varieties of Walnuts	Kabul - Mandawi	AF	£69:6-52 2999	x	x	х		6.68	х				11.43	Trace	< LOD	< LOD	
	502	591	WN06-PR-51-10-28-2015-502-	Walnut - WN06	Parwan	Other varieties of Walnuts		AF												L	'	< LOD	
8	86	592	WN04-S-S1-10-31-2015-086-	Walnut - WN04	Samangan	Korak Walnuts (opening in shell)		AF	1											Trace	< LOD	< LOD	
40/17/2015	409	593	WN05-KBL-S1-10-10-15-409-006	Walnut - WN05	Kabul	Kaghazi Walnuts (paper shelled)	Kabul -	AF	Lat: 34,30539199 Long: 69.10345700	x	x	х		7.10	х				<lod< td=""><td>8.21</td><td>< LOD</td><td></td><td></td></lod<>	8.21	< LOD		
10/17/2015 4	438	594	WN01-K8L-S1-10-10-15-438-003	Walnut - WN01	Kabul	Zard Walnuts (yellow kernels)	Kabul, Mandawi	AF	N34;30;54,3899 E69:10:35.1099	×	x	х		7.60	х	I T			< LOD	< LOD	< LOD	< LOD	
10/5/2015 3	320	595	WN05-KBL-S1-10-3-15-320-002	Walnut - WN05	Kabul	Kaghazi Walnuts (paper shelled)	Kabul - Mandawi	AF	N34;30;55,6499	x	x	х		7.15	х				41.40	10.50	< LOD	< LOD	
10/17/2015 3	373	595	WN05-K8L-S1-10-4-15-373-005	Walnut - WN05	Kabul	Kaghazi Walnuts (paper shelled)	Kabul, Mandawi	AF	N34;30;54,7200	x	x	х		6.80	х				< LOD	Trace	< LOD	< LOD	
	71	597	WN05-S-S1-10-31-2015-071-	Walnut - WN05	Samangan	Kaghazi Walnuts (paper shelled)		AF	E69:10:34.5299													< LOD	
	145	598	WN04-S-S2-11-1-2015-145-	Walnut - WN04	Samangan	Korak Walnuts (opening in shell)		AF												8.60	< LOD	< LOD	
		598 N					+		+	<u>├ </u>										8.00			+
	56	N	WN05-S-S2-10-30-2015-056-	Walnut - WN05	Samangan	Kaghazi Walnuts (paper shelled)	+	AF	+	├											'		+
	1		WN01-8-S1-10-6-15-001-001	Walnut - WN01	Balkh	Zard Walnuts (yellow kernels)	Baikh -	AF	N/Av	x	x	х		7.65	х				< LOD	L	'		1
	8		WN06-H-S4-8-12-15-008-013	Walnut - WN06	Herat	Other varieties of Walnuts	Herat -	AF	Lat: 34204008999 Long: 62.112619000	×	x	х		6.85	х				Trace	L			
11/14/2015 5	54		WN01-K8L-S-1-10-15-054-008	Walnut - WN01	Kabul	Zard Walnuts (yellow kernels)		AF	Lat: 36,15787 Long: 068,01236	x	x	х		6.83	х				11.29				
10/17/2015	73		WN05-S-S1-8-4-15-073-010	Walnut - WN05	Samangan	Kaghazi Walnuts (paper shelled)	Dry fruit market, Mazar	AF	N36;32;13,660 E67:7:31.6499	x	x	х		7.42	х				< LOD	1		. —	
1/14/2015 5	578		WN01-K8L-S1-10-11-15-578-007	Walnut - WN01	Kabul	Zard Walnuts (vellow kernels)		AF	Lat: 35.0505800	x	x	х		6.95	x				21.75	1			1

	Date of Die Seis, Randion Sample D Commoditi			npie Log Sampling Centrol Log Elias No Yes No Yes	Control Log pH May No Afghanistan Afataain 6.63 Y			UNL UNL GGW UNL Rectar Boster Aflatasin ppb Aflatosis ppb Aflatosis ppb		Austria Afghanista Borner Zearaiencone ppb DON ppb		Italy Italy Catrines Construints DOM ppb NIV ppb NEO ppb						UNL UNL GGW Rozner Baster T2/HT2 ppb T2/HT2 ppb T.	UNL UNL GGW incom Nooran WT2 ppb T2/HT2 ppb	Т2/нт2 ppb Т2/нт2	ochristown ppb Convis	INL UNL GOW Roar Boart Lowin ppb Ochratoxin ppb	Ochratoxin ppb	KSU VICAM Ochratowin ppb Oc	UNL Austria Nessen Chratevin ppb Ochratevin ppb
	N/26/2015 78 2 WE2-8-53-8-3-15-028-007 Four-WE2 N/15/2015 87 3 W62-8-53-3-6-15-028-007 Four-WE2	Initial State and a state of the state	AF N36(272050 X AF N36(272050 X E66.974201 X AF N36(-02-66.9699 X	x x x x x x x x x	7.37 X	x x x x x x	Trace <lod< td=""><td></td><td><100 <100</td><td><100 <100 <100 3500.00</td><td><100</td><td><100 <100 <100</td><td>Trace Trace</td><td><100</td><td>23.70 Trace</td><td><100 <100 <10 <100 <100 <10</td><td> <l00< li=""> <l00< li=""> </l00<></l00<></td><td>< 100</td><td>38.25 36.95</td><td><100 <10 <100 <10</td><td>< 100</td><td>4.48</td><td><100</td><td>3.02</td><td><100</td></lod<>		<100 <100	<100 <100 <100 3500.00	<100	<100 <100 <100	Trace Trace	<100	23.70 Trace	<100 <100 <10 <100 <100 <10	 <l00< li=""> <l00< li=""> </l00<></l00<>	< 100	38.25 36.95	<100 <10 <100 <10	< 100	4.48	<100	3.02	<100
	9/14/2025 43 5 W02-H-53-8-10-15-043-005 Wheat - W01 9/29/2025 30 6 W07-H-55-8-15-030-006 Flour - W07	Herat Asiabs NII Flour Of Alghan Origin (Wheat offen Herat - Herat - Herat - Honora bakers in each regions (Ether dirt or cement Herat - Herat - Herat -	PA 10 18864	x x x x x x x x x x x		x x x x x x x	9.60	< 100	<100 <100	<lod <lod="" td="" trace<=""><td><100</td><td><100 <100 <100</td><td><100</td><td><100 70.10</td><td>Trace</td><td></td><td><100</td><td>< 100</td><td>77.60</td><td><100 <10 <100 <10</td><td><.00</td><td>3.09 <100</td><td><100</td><td><100</td><td></td></lod>	<100	<100 <100 <100	<100	<100 70.10	Trace		<100	< 100	77.60	<100 <10 <100 <10	<.00	3.09 <100	<100	<100	
	8 W08-8-53-8/4/2015-057-002	Auber Noor Australier of Metern Cristin Wheat often	AF LLC: 34,22136420 X LLC: 34,22136420 X LLC: 47,2136420 X AF N34,23452 X	x x 	7.30 X	x x x	13.90	< LOD		Trace <100 270.00	<ldd <ldd <ldd< td=""><td><lod <lod="" <lod<="" td=""><td><100 <100 <100</td><td><100 <100 51.22</td><td><lod <lod <lod< td=""><td><000 <00 <00 <00 <00 <00 <00 <00 <00 <0</td><td></td><td><100</td><td>41.85</td><td><10</td><td></td><td>< 100</td><td></td><td></td><td><100</td></lod<></lod </lod </td></lod></td></ldd<></ldd </ldd 	<lod <lod="" <lod<="" td=""><td><100 <100 <100</td><td><100 <100 51.22</td><td><lod <lod <lod< td=""><td><000 <00 <00 <00 <00 <00 <00 <00 <00 <0</td><td></td><td><100</td><td>41.85</td><td><10</td><td></td><td>< 100</td><td></td><td></td><td><100</td></lod<></lod </lod </td></lod>	<100 <100 <100	<100 <100 51.22	<lod <lod <lod< td=""><td><000 <00 <00 <00 <00 <00 <00 <00 <00 <0</td><td></td><td><100</td><td>41.85</td><td><10</td><td></td><td>< 100</td><td></td><td></td><td><100</td></lod<></lod </lod 	<000 <00 <00 <00 <00 <00 <00 <00 <00 <0		<100	41.85	<10		< 100			<100
	9/22/2015 40 10 WE3+53-610-55 040-004 Flour- WE2 9/24/2015 206 11 WE7+55-96-526-002 Flour- WE2 9/24/2015 206 12 WE9+85-14-10-15-248-002 Flour- WE9	Hend Control Million of Angliban Chiglio Jouandy in a remer floor and Angliban Chiglio Jouandy in a remer floor and conclusion. Hend, Carlo Million Hend Two max Malaries in such regions (Ether dirt or centent floor. In sea summer link). Hend, Carlo Million Kondale Palatzan floor and Nabel -	AF N 42,41257 X 1621106427 X 1621106427 X 9K 1621126427 X 1000000000000000000000000000000000000	x x x x x x	7.18 X 7.07 X 7.31 X	x x x x x x x x	9.85	< LOD < LOD < LOD	<000 <000 <000 <000 <000 <000	0.67 Trace <lod 600.00<br=""><lod< td=""><td><100 <100 <100</td><td><100 <100 <100</td><td><lod Trace Trace</lod </td><td><100</td><td><lod <lod <lod< td=""><td><100 <100 <10 <100 <100 <10 <100 <100 <1</td><td>< 100</td><td>< 100</td><td>65.70</td><td><100 <10</td><td>< LDD</td><td>< 100</td><td><100</td><td><100</td><td><100</td></lod<></lod </lod </td></lod<></lod>	<100 <100 <100	<100 <100 <100	<lod Trace Trace</lod 	<100	<lod <lod <lod< td=""><td><100 <100 <10 <100 <100 <10 <100 <100 <1</td><td>< 100</td><td>< 100</td><td>65.70</td><td><100 <10</td><td>< LDD</td><td>< 100</td><td><100</td><td><100</td><td><100</td></lod<></lod </lod 	<100 <100 <10 <100 <100 <10 <100 <100 <1	< 100	< 100	65.70	<100 <10	< LDD	< 100	<100	<100	<100
	9/29/2015 1 13 W02-H-51-8-9-15-001-009 Flour - W02	Herat Gritt Mill Flour of Alghan Crigin (usually in a Herat - cement floor and structure)	AF IN3625423000 X AF Lorg (35302000 X Lorg (35332000 X AF IN362453 AF IN562500 Y	x x x x x x	6.34 X	x x x x x	4.38	< 100	<100 <100	<100 <100 1290.00	<100	<100 <100 <100	Trace	<100	<lod <lod <lod< td=""><td><100 <100 <10 <100 <100 <10 <100 <100 <1</td><td>< 100</td><td><100</td><td>83.70</td><td><100 <10</td><td></td><td>< 100</td><td><100</td><td>< 100</td><td><100</td></lod<></lod </lod 	<100 <100 <10 <100 <100 <10 <100 <100 <1	< 100	<100	83.70	<100 <10		< 100	<100	< 100	<100
	9/22/2015 86 17 W02-H-53-6-11-15-086-003 Flour - W02	Herst Grist Mill Four of Afghan Origin (usually in a Herst Court Mill Four of Afghan Origin (usually in a Kennet Floor and Kityschen)	AF EEE 12465099 III AF EEE 1246509 III AF EEE 1246509 X AF EEE 1246509 X		7.35 X 7.20 X		22.78	< 100	<100 <100	Trace <lod td="" trace<=""><td><lod <lod< td=""><td><100 <100 <100</td><td><100</td><td>92.78 <100</td><td><100</td><td><100 <100 <10 <100 <100 <10</td><td>< 100</td><td>Trace</td><td>86.10 64.60</td><td><100 <10</td><td>1.62</td><td>< 100</td><td><100</td><td><100</td><td><100</td></lod<></lod </td></lod>	<lod <lod< td=""><td><100 <100 <100</td><td><100</td><td>92.78 <100</td><td><100</td><td><100 <100 <10 <100 <100 <10</td><td>< 100</td><td>Trace</td><td>86.10 64.60</td><td><100 <10</td><td>1.62</td><td>< 100</td><td><100</td><td><100</td><td><100</td></lod<></lod 	<100 <100 <100	<100	92.78 <100	<100	<100 <100 <10 <100 <100 <10	< 100	Trace	86.10 64.60	<100 <10	1.62	< 100	<100	<100	<100
	W2/V2025 #A 1B W00-46-26-36-35-056-080-001 HOD - W00 5/14/2025 5:1 1:9 W02-952-86-301 Hod - W20 220 2:0 W00 H00-16-22-86-315-021 Wheet - W20	I BARD Records and the formation of the second seco	AF Lat: 34.18216709 X	X X X	6.87 X	x x x	5.97	< LOD < LOD	<100 <100	<100 405.00	320.00			75.40	<lod< td=""><td>52.22 <1.00 75.</td><td>0 < LOD</td><td>110.95</td><td>40.70</td><td><100 75.0</td><td>2.10</td><td>< 100</td><td><100</td><td>1.75 <1.00</td><td><100</td></lod<>	52.22 <1.00 75.	0 < LOD	110.95	40.70	<100 75.0	2.10	< 100	<100	1.75 <1.00	<100
	9/21/2015 66 23 W05-8-53-9-54-15-066-021 Flour - W05	Balish Avabed on Sirt San recommended Su shart Start or Balish Avabed on Sirt Floor next to a stream stored on Sirt Floor next to a stream	AF N36;42;3,2000	x x x	7.89 X	x x x	<100		<100	<lod Trace</lod 	<100	<100 <100 <100	Trace	102.71 <1.00	<lod< td=""><td>55.26 <10</td><td>< 100</td><td><100 <100</td><td>26.65 57.10 < LOD 34.75</td><td><10</td><td>2.91</td><td>2.80 <100 100 <100</td><td><100</td><td>4.46 6.96</td><td></td></lod<>	55.26 <10	< 100	<100 <100	26.65 57.10 < LOD 34.75	<10	2.91	2.80 <100 100 <100	<100	4.46 6.96	
N N N N N N N N N N N N N N N N N N N N N N	205 25 W05-8N-52-9-8-15-0205-	Kandabar Asiaba Mil Flour of Aglan Origin (Wheat often transformed on dirt floor met to a tream)	AF	x x	7.54 X	x x	<100	<100 <100 <100	<100	Trace <100	<ldd Trace <ldd< td=""><td><100 <100 <100 <100 <100 <100 <100 <100</td><td>Trace < LOD Trace</td><td><100</td><td><lod< td=""><td>69.02 <10</td><td>> <100 > <100 > <100</td><td><100 <100</td><td><100 51.55</td><td><10</td><td></td><td>3.04 < 100</td><td><100</td><td>5.53</td><td><100</td></lod<></td></ldd<></ldd 	<100 <100 <100 <100 <100 <100 <100 <100	Trace < LOD Trace	<100	<lod< td=""><td>69.02 <10</td><td>> <100 > <100 > <100</td><td><100 <100</td><td><100 51.55</td><td><10</td><td></td><td>3.04 < 100</td><td><100</td><td>5.53</td><td><100</td></lod<>	69.02 <10	> <100 > <100 > <100	<100 <100	<100 51.55	<10		3.04 < 100	<100	5.53	<100
	9(21/2005 64 27 W00-9-53-9-11-15-064-022 Pour-W00 9(15/2005 99 28 W00-9-53-9-55-099-006 Pour-W00	Balleh kalab Mil Flour of Aghan Origin (Meas often steerd on dir floar net to a dream) Balleh kalab Mil Flour of Aghan Origin (Meas often Balleh kalab Mil Flour of Aghan Origin (Meas often steerd on dir floar net to a dream) Balleh kalab Mil Floar oft to a dream	A5 647.124.05090 X	x x x x x	7.64 X 7.62 X	x x x x x	<100			Trace < LOD	<100 <100 <100	<100 <100 <100	Trace	123.75 <100	<100	56.49 <10	0 <100 <100 <100	<100	32.40 \$7.55	<10		2.65 < LOD	<100	2.79	
	9/28/2015 84 30 W07-8-53-8-3-15-084-003 Flour-W07	Balleh Two naan bakeries in each regions (Either dirt or cement Balleh, Police head quarter 5 Roore was avenue itTel	AF N36;43;50,2200 X FE1:5:55,32300 AC N36;45;7,3200 Y	x x	6.43 X	x x x	10.98 Trace			Trace	<100			48.91 <100				<lod td="" trace<=""><td>< 100 92.10 76.35</td><td><10</td><td><100</td><td>5.57 < LOD < LOD</td><td></td><td>5.31</td><td></td></lod>	< 100 92.10 76.35	<10	<100	5.57 < LOD < LOD		5.31	
	34	Anne experience of the second	6572114300 A		2.487 A		120	Trace/ <lod <="" <lod<="" lod="" td="" trace=""><td><100</td><td>114.9</td><td><100</td><td><100 <100 <100 <100 <100 <100 <100 <100</td><td>Trace</td><td> <l00 <l00<="" li=""> </l00></td><td><100</td><td>44.87 <10</td><td>< 100</td><td><100/<100</td><td>126.15 10/<100 81.90</td><td><10</td><td>24</td><td><100 <100 <100</td><td><100</td><td></td><td></td></lod>	<100	114.9	<100	<100 <100 <100 <100 <100 <100 <100 <100	Trace	 <l00 <l00<="" li=""> </l00>	<100	44.87 <10	< 100	<100/<100	126.15 10/<100 81.90	<10	24	<100 <100 <100	<100		
	6/22/2025 BB 25 WID: 4-54-6-3-15-088-028 Without - W01 6/26/2025 2.8 3.6 WID: 4-56-8-5-15-028-007 Floar - W07 9/15/2025 2.8 3.6 WID: 4-56-85-85-020-0011 Whest - W01 9/15/2025 9.2 3.7 WID: 4-56-85-85-020-0011 Whest - W01	autori interview of their rent to a stream interview of their rent interview of their ren	N (0), 0, 0, 000 X 647 - 148 (2017) X 9% Loss (2017) X 1000 (2011) 1054 (2000) X 4AF N (2012) X 647 + 148 (2001) X X	x x x x x x	7.55 X 6.90 X 7.64 X	x x x x x x	26.41 8.45 <100	<000 <000 <000 <000 <000	<000	Trace	<l00 <l00 <l00< td=""><td><100 <100 <100 <100 <100 <100 <100 <100 <100</td><td><100 Trace</td><td><100 218.09 <1.00 106.52 <1.00</td><td><lod< td=""><td>60.94 < 10 63.20 < 10</td><td>< 100</td><td><lod <lod="" <lod<="" td=""><td><100 71.25 <100 49.55</td><td><10</td><td></td><td>LOD < LOD</td><td><100 <100 <100</td><td>1.93 < LOD</td><td></td></lod></td></lod<></td></l00<></l00 </l00 	<100 <100 <100 <100 <100 <100 <100 <100 <100	<100 Trace	<100 218.09 <1.00 106.52 <1.00	<lod< td=""><td>60.94 < 10 63.20 < 10</td><td>< 100</td><td><lod <lod="" <lod<="" td=""><td><100 71.25 <100 49.55</td><td><10</td><td></td><td>LOD < LOD</td><td><100 <100 <100</td><td>1.93 < LOD</td><td></td></lod></td></lod<>	60.94 < 10 63.20 < 10	< 100	<lod <lod="" <lod<="" td=""><td><100 71.25 <100 49.55</td><td><10</td><td></td><td>LOD < LOD</td><td><100 <100 <100</td><td>1.93 < LOD</td><td></td></lod>	<100 71.25 <100 49.55	<10		LOD < LOD	<100 <100 <100	1.93 < LOD	
	9/29/2015 251 38 W02-0N-51-9-10-15-251-003 Flour - W02	Randahar senert floar and structurel for the floar and structurel floar and structurel floar and structurel floar and structurel floar and structure floar	PX Lize 23,227360 X Lizer 52,273160 X TX Lize 34,2010930 X Lizer 52,1752200 X AF N35-03,2300	x x x x x x x x	6.60 X 6.47 X 7.70 X	x x x x x x x x	9.05 Trace <1.00	<00 <00 <00 <00 <00 <00 <00 <00 <00 <00	<100 <100 <100	Trace Trace	<100 <100 <100	<100 <100 <100 <100 <100 <100 <100 <100	<lod Trace <lod< td=""><td></td><td><lod <lod <lod< td=""><td></td><td>0 <100 <100 <100 <100</td><td><lod <lod<="" td=""><td>< 100 83.20</td><td></td><td></td><td>4.09 < 1.00 3.22 < 1.00 < 1.00</td><td><100 <100 <100</td><td></td><td></td></lod></td></lod<></lod </lod </td></lod<></lod 		<lod <lod <lod< td=""><td></td><td>0 <100 <100 <100 <100</td><td><lod <lod<="" td=""><td>< 100 83.20</td><td></td><td></td><td>4.09 < 1.00 3.22 < 1.00 < 1.00</td><td><100 <100 <100</td><td></td><td></td></lod></td></lod<></lod </lod 		0 <100 <100 <100 <100	<lod <lod<="" td=""><td>< 100 83.20</td><td></td><td></td><td>4.09 < 1.00 3.22 < 1.00 < 1.00</td><td><100 <100 <100</td><td></td><td></td></lod>	< 100 83.20			4.09 < 1.00 3.22 < 1.00 < 1.00	<100 <100 <100		
	9/29/2015 26 42 W09-H-53-8-9-15-026-001 Flour - W09	Balich Balich Balich, Shrashad Dehdadi Herat Pakistan Sour	PK Lat: 34,2010600 X PK Lat: 34,2010600 X	x x x x x x	6.00 X 6.70 X 7.26 X	x x x x x x	<100 9.97 5.32	< LOD < LOD < LOD	<100	< LOD Trace	<ldd <ldd <ldd< td=""><td><100 <100 <100</td><td>Trace</td><td>Trace 25.14</td><td><100</td><td>99.26 <10</td><td>< 100</td><td><100</td><td><100 <100</td><td><10</td><td>10.81</td><td>3.60 < 1.00</td><td><100</td><td>2.91</td><td></td></ldd<></ldd </ldd 	<100 <100 <100	Trace	Trace 25.14	<100	99.26 <10	< 100	<100	<100 <100	<10	10.81	3.60 < 1.00	<100	2.91	
	8/28/2015 250 44 W02-8N-52-9-10-15-250-005 Flour - W02	Kundahar Grint Mill Rour of Alghan Crigin (Jusually) is a remark Provide and interview and interview and the second seco	AF Lat: 31,8023639 X Inner 65,4703120 X AF N8,802,52000 X ESE 6,4723200 X AF N8,20251,6276,8274292 X	x x x x x x	7.23 X 7.35 X 7.35 X	x x x x x x	5.90 Trace Trace	Trace < LOD < LOD Trace < LOD	<100 <100 <100	Trace	<100 <100 <100				Trace < LOD < LOD	27.23 < 10 28.06 < 10 Trace < 10	0 <100 <100 <100	<lod <lod="" <lod<="" td=""><td></td><td><10</td><td></td><td>inace < LOD</td><td><100 <100 <100</td><td></td><td></td></lod>		<10		inace < LOD	<100 <100 <100		
N N N N N N N N N N N N N N N N N N N N N N N N	9/22/2015 82 48 W02-8-53-8-3-15-082-001 Flour-W02	Balloh Asaloh MB Flour of Alghun Origin (Nivers of Annes) Balloh - Istando and Ki Gaor and Ka a Asama) Balloh - Balloh Grint MB Flour of Alghun Origin (Jusanily in a center of Brour of Alghun Origin (Jusanily in a center of Brour of Alghun Origin (Jusanily in a Balloh, Police Iwad quarter 5	AF N/Av AF N/Av AF NSA/82,01,0000 XF SE7.124.05999 AF Ltt: 34,2122999	x x x x x x x x x x x x x x x x x x x	7.36 X		Trace Trace	 <l00< li=""> <li< td=""><td><100 <100 <100</td><td>360.00 Trace</td><td><l00 <l00 <l00< td=""><td><lod <lod="" <lod<="" td=""><td>Trace</td><td><100 <100</td><td><lod Trace Trace</lod </td><td>Trace <10</td><td>0 <100 0 <100 0 <100</td><td>Trace <100 001> 001> Trace 400</td><td>< LOD < LOD < LOD < LOD</td><td><10</td><td>Trace</td><td>2.20 <1.00 2.48 <1.00</td><td><100</td><td>5.67</td><td></td></lod></td></l00<></l00 </l00 </td></li<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<></l00<>	<100 <100 <100	360.00 Trace	<l00 <l00 <l00< td=""><td><lod <lod="" <lod<="" td=""><td>Trace</td><td><100 <100</td><td><lod Trace Trace</lod </td><td>Trace <10</td><td>0 <100 0 <100 0 <100</td><td>Trace <100 001> 001> Trace 400</td><td>< LOD < LOD < LOD < LOD</td><td><10</td><td>Trace</td><td>2.20 <1.00 2.48 <1.00</td><td><100</td><td>5.67</td><td></td></lod></td></l00<></l00 </l00 	<lod <lod="" <lod<="" td=""><td>Trace</td><td><100 <100</td><td><lod Trace Trace</lod </td><td>Trace <10</td><td>0 <100 0 <100 0 <100</td><td>Trace <100 001> 001> Trace 400</td><td>< LOD < LOD < LOD < LOD</td><td><10</td><td>Trace</td><td>2.20 <1.00 2.48 <1.00</td><td><100</td><td>5.67</td><td></td></lod>	Trace	<100 <100	<lod Trace Trace</lod 	Trace <10	0 <100 0 <100 0 <100	Trace <100 001> 001> Trace 400	< LOD < LOD < LOD < LOD	<10	Trace	2.20 <1.00 2.48 <1.00	<100	5.67	
	9/29/2015 249 50 W02-00-53-9-10-15-249-006 Rour-W02 9/15/2015 85 51 W02-9-33-9-15-0085-009 Rour-W02	Kandahar Gritz Mill Flour of Afghan Origin (aussily in a Kandahar - Darran District Basileh Aslada Mill Flour of Afghan Origin (Wheat often Basileh, Shenbad Dehdad	LOW SO AVYION	x x x x		x x x x	6.46 <lod< td=""><td><100 <100 <100 <100 4.17 <100 <100</td><td><l00 <l00< td=""><td>445.00</td><td><l00 <l00< td=""><td><100 <100 <100</td><td></td><td>30.15 <1.00</td><td></td><td></td><td></td><td></td><td><100 <100 <100 <100</td><td><10</td><td>Trace</td><td>100 < 100</td><td><100</td><td>8.34 9.45</td><td></td></l00<></l00 </td></l00<></l00 </td></lod<>	<100 <100 <100 <100 4.17 <100 <100	<l00 <l00< td=""><td>445.00</td><td><l00 <l00< td=""><td><100 <100 <100</td><td></td><td>30.15 <1.00</td><td></td><td></td><td></td><td></td><td><100 <100 <100 <100</td><td><10</td><td>Trace</td><td>100 < 100</td><td><100</td><td>8.34 9.45</td><td></td></l00<></l00 </td></l00<></l00 	445.00	<l00 <l00< td=""><td><100 <100 <100</td><td></td><td>30.15 <1.00</td><td></td><td></td><td></td><td></td><td><100 <100 <100 <100</td><td><10</td><td>Trace</td><td>100 < 100</td><td><100</td><td>8.34 9.45</td><td></td></l00<></l00 	<100 <100 <100		30.15 <1.00					<100 <100 <100 <100	<10	Trace	100 < 100	<100	8.34 9.45	
	9/15/2015 45 54 W02-H55-08-F5-08 Flour-W02	Automini Born, see sanai Dal Balah. Auto Will Foor Af glann Origin (Wheat often Balah, Shenbad Dehdud Auto Mill Foor and Na unream) Henzt Autob Mill Foor and Na unream) Henzt - Auto Mill Foor and Na Unream)	AF 1131/15000 X AF 133(33453 X	x x x x	7.63 X 6.60 X	x x x	Trace < LOD	<100 528 <100 <100 432 <100 <100	<100	Trace	<l00 <l00 <l00< td=""><td><lod <lod="" <lod<="" td=""><td></td><td></td><td><100</td><td>Trace < L0</td><td></td><td></td><td>49.35 < LOD 30.20 < LOD</td><td><10</td><td>· · · · · · · · · · · · · · · · · · ·</td><td>2.97 < LOD</td><td>2.79</td><td>5.79 5.49</td><td></td></lod></td></l00<></l00 </l00 	<lod <lod="" <lod<="" td=""><td></td><td></td><td><100</td><td>Trace < L0</td><td></td><td></td><td>49.35 < LOD 30.20 < LOD</td><td><10</td><td>· · · · · · · · · · · · · · · · · · ·</td><td>2.97 < LOD</td><td>2.79</td><td>5.79 5.49</td><td></td></lod>			<100	Trace < L0			49.35 < LOD 30.20 < LOD	<10	· · · · · · · · · · · · · · · · · · ·	2.97 < LOD	2.79	5.79 5.49	
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	8 134 W02-KBL-51-11-17-15-008-	Kabul Grint MI Pour Al Applan Criging Jussially in a carrier floor and structurel Vabul Grint MI Pour of Alghan Crigin Jussially in a carrier floor and structurel carrier floor and structurel	AS AS					< 100 < 100	<100 <100	0.22	<100	<lod <lod="" <lod<="" td=""><td>< LOD</td><td><100</td><td></td><td>Trace <100 <10</td><td>> <100</td><td><100</td><td>< 100</td><td><100 <10</td><td></td><td>100</td><td><100 <100 <100</td><td>4.59 3.91 5.4</td><td><000 <000 <000 <000 <000 <000</td></lod>	< LOD	<100		Trace <100 <10	> <100	<100	< 100	<100 <10		100	<100 <100 <100	4.59 3.91 5.4	<000 <000 <000 <000 <000 <000
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5 141	W22-881-52-11-16-15-005-	Kabal Griz MB Rour of Alghan Origin (assily in a remote Rour and rithing handle) in a	AF					<100	1	<100 <100	<100	<100	<100 <100	<100	< 109	<100	<100	 34.51 <100 <1	0 <100	<lod< th=""><th>Toce</th><th><100 <100</th><th><100</th><th><100</th><th>62 <100</th><th><100</th></lod<>	Toce	<100 <100	<100	<100	62 <100	<100
13 142 2 143	W02-68L-52-11-17-15-013- W02-68L-51-11-16-15-002-	Kabul Gritt Mill Flour of Afghan Crigin (assally in a center floor and structure)	46 46					<000		<100 <100		<100	<100 <100 <100 <100	<100	< 100	<100	<100	29.17 2.04 29.17 2.04 100 <li< td=""><td>0 <100 0 <100</td><td></td><td><100</td><td>2.04 <1.00 <1.00 <1.00</td><td><100</td><td>(100</td><td>2.99 5.29 <100</td><td><100</td></li<>	0 <100 0 <100		<100	2.04 <1.00 <1.00 <1.00	<100	(100	2.99 5.29 <100	<100
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608 149	W12-69-51-11-17-15-608-	Kapisa Gritt Mil Floar of Afghan Crigin (assaily in a reament Floar and structural Kapisa Gritt Mil Floar of Afghan Crigin (assaily in a reament Floar and structural	Al Al					<000 < 100		<000 <000 <000 <000		<100	<100 <100 <100 <100	<100 <100 <100	< 100	Trace <1.00	<100	22.52 <l00< td=""> <l0< td=""> Trace <l00< td=""> <l0< td=""> Trace <l00< td=""> <l0< td=""> Trace <l00< td=""> <l0< td=""></l0<></l00<></l0<></l00<></l0<></l00<></l0<></l00<>	0 <100 0 <100	<lod Trace</lod 	30.30 29.40	<100 <100 <100 <100	< 100	<100	4.33 <100 7.38 <100	<100
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595 158 588 159	W02-8P-51-11-16-15-0595 W01-8P-51-11-16-15-0588	Kapika Grite MII Flour of Afghan Crigin Jouanly in a remort Flour and thracknell Kapika Akiaba MII Flour of Afghan Origin (Wheat offer Ropika Grite MII Flour of Afghan Crigin Jouanly in a remort Flour and thrack stream).	AF					<100		<100 <100 <100 <100	<100	<100				<100 <100 <100	<100	28.15 <1.00 <1/2	< 100	Trace	40.70	<100 <100	<100	<100	4.09 5.37	<100 <100 <100
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11/3/2005 542 194	W07-5-52-11-1-15-142-023	Flour-WD7 Samangan Roors see samale IDs) Flour-WD9 Parwan Pakistan flour	Parwan, Charkar city PK	N 36:16:022	x x		x 7.08 X X x 6.53 X X	x x 6.57 520 x x 5.45 4.65		<100 <100 <100 <100 <100 <100	<100	<l00 <l00 trace<br=""><l00 td="" trace<=""><td><100 <100</td><td><100</td><td>< 100</td><td><l00 <l00 <l00< td=""><td><000 Trace <000 <000 Trace</td><td> 122.01 <100 <10 64.26 <100 <10 78.66 <100 <10 25.90 <100 <10</td><td>0 <100 0 <100 0 /100</td><td><lod <lod <lod< td=""><td>71.60 <100 47.00</td><td><100 <100 <100 <100 Trace <100 <100 32.18</td><td><100</td><td><100</td><td>1.74 1.38 4.43</td><td><100 0.97 <100</td></lod<></lod </lod </td></l00<></l00 </l00 </td></l00></l00></l00 	<100 <100	<100	< 100	<l00 <l00 <l00< td=""><td><000 Trace <000 <000 Trace</td><td> 122.01 <100 <10 64.26 <100 <10 78.66 <100 <10 25.90 <100 <10</td><td>0 <100 0 <100 0 /100</td><td><lod <lod <lod< td=""><td>71.60 <100 47.00</td><td><100 <100 <100 <100 Trace <100 <100 32.18</td><td><100</td><td><100</td><td>1.74 1.38 4.43</td><td><100 0.97 <100</td></lod<></lod </lod </td></l00<></l00 </l00 	<000 Trace <000 <000 Trace	 122.01 <100 <10 64.26 <100 <10 78.66 <100 <10 25.90 <100 <10	0 <100 0 <100 0 /100	<lod <lod <lod< td=""><td>71.60 <100 47.00</td><td><100 <100 <100 <100 Trace <100 <100 32.18</td><td><100</td><td><100</td><td>1.74 1.38 4.43</td><td><100 0.97 <100</td></lod<></lod </lod 	71.60 <100 47.00	<100 <100 <100 <100 Trace <100 <100 32.18	<100	<100	1.74 1.38 4.43	<100 0.97 <100
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20 107 27 198 202 199	W02-5-52-11-1-2015-0102-	Samangan Buor aan daaxees in each regions jutteer oor or centern Boors was sensel in the Samangan Gritt MII Roar of Afghan Chrigin (auailty in a reamore Roors and stront snall	12					429		<100 <100 <100 <100	<lod< td=""><td><100</td><td><lod <lod<br=""><lod <lod<="" td=""><td><000 <000 <000 <000</td><td>< 100</td><td><100 <100</td><td><lod Trace</lod </td><td></td><td>0 <100 0 <100</td><td><ldd <ldd Trace 48.29</ldd </ldd </td><td>31.00 54.80</td><td><100 <100 <100 <100</td><td><100</td><td></td><td><1.00 4.19</td><td>1.80 <1.00 <1.00</td></lod></lod></td></lod<>	<100	<lod <lod<br=""><lod <lod<="" td=""><td><000 <000 <000 <000</td><td>< 100</td><td><100 <100</td><td><lod Trace</lod </td><td></td><td>0 <100 0 <100</td><td><ldd <ldd Trace 48.29</ldd </ldd </td><td>31.00 54.80</td><td><100 <100 <100 <100</td><td><100</td><td></td><td><1.00 4.19</td><td>1.80 <1.00 <1.00</td></lod></lod>	<000 <000 <000 <000	< 100	<100 <100	<lod Trace</lod 		0 <100 0 <100	<ldd <ldd Trace 48.29</ldd </ldd 	31.00 54.80	<100 <100 <100 <100	<100		<1.00 4.19	1.80 <1.00 <1.00
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214 902 215 904 216	W01-8-52-5-8-15-0302- W01-8-54-5-8-15-0304-	Wheat - WO1 Balikh Asiabs MII Flour of Aglian Origin (Wheat often stored on dirt floar owst to a stream) Wheat - WO1 Balikh Asiabs MII Flour of Aglian Origin (Wheat often	Rallih, Nawabad, Dehdadi AF Rallih, Qandahari masjid, Dehdadi AF	N3636(7,3500 667/7114200 N3636(3,3200				Trace Trace Trace		<100 <100 <100 <100 <100 <100			<l00 <l00<br=""><l00 <l00<br=""><l00 <l00<="" td=""><td><100 <100 <100 <100 <100</td><td>< 100</td><td><lod <lod <lod< td=""><td>Trace Trace 23.10</td><td>8224 < U 7150 < U 97.13 < U</td><td><100</td><td><lod <lod 52.05</lod </lod </td><td>42.95 63.55 55.50</td><td><100 <100 <100</td><td><000 <000 Trace</td><td><100</td><td>4.33 4.2 6.02</td><td></td></lod<></lod </lod </td></l00></l00></l00>	<100 <100 <100 <100 <100	< 100	<lod <lod <lod< td=""><td>Trace Trace 23.10</td><td>8224 < U 7150 < U 97.13 < U</td><td><100</td><td><lod <lod 52.05</lod </lod </td><td>42.95 63.55 55.50</td><td><100 <100 <100</td><td><000 <000 Trace</td><td><100</td><td>4.33 4.2 6.02</td><td></td></lod<></lod </lod 	Trace Trace 23.10	8224 < U 7150 < U 97.13 < U	<100	<lod <lod 52.05</lod </lod 	42.95 63.55 55.50	<100 <100 <100	<000 <000 Trace	<100	4.33 4.2 6.02	
128 217 89 218	W05-5-51-11-1-2015-128- W01-8-53-3-8-15-089-	Wind: Back Advances of the out of Specific spectrum of the spectrum o	AF Ballity, Shenabad Dehdad AF	E577:114200 N36;45;3,2200 E57:15:61999 N36;46;32,2600 E57:2:10:51999				Trace		<100 <100 <100 <100			<ldd <ldd<br=""><ldd <ldd<="" td=""><td><100</td><td></td><td><100</td><td>Trace Trace</td><td>105.84 < b 83.51 < b</td><td>o <100</td><td><100</td><td>40.40</td><td><100</td><td>< 100</td><td><100</td><td>3.1 4.98</td><td></td></ldd></ldd>	<100		<100	Trace Trace	105.84 < b 83.51 < b	o <100	<100	40.40	<100	< 100	<100	3.1 4.98	
61 220	W01-0-53-3-0-15-086- W04-0-51-0-4-2015-065-	Wheat - W01 Balleh Aslabs MII Flour of Aghan Grigin (Wheat often thoredon dist for next to a stream) Balleh Aslabs and Grist MII Flour of Uzbekistan Origin Median Dustru Low Gene Genetics Balleh (Modelin sh	Raikh, Shenshad Dehdad AF UZ ade AF					4.26 Trace		<100 <100 <100 <100			<100 <100 <100 <100	<1.00 <1.00 <1.00	< 100	<lod Trace</lod 	30.02 21.94		0 <100 0 <100	Trace 77.23 70.12	61.00 42.70	<100	<100		3.85 <1.00	
225 258 9/12/2005 10 259	807-KN -53-9-10-2015-235- 807-H 52-9-9-15-010-002	Raisins - R07 Kandahar Sun dried Tayefee (name for the variety from the control & Buhan Sun dried Tayefee (name for the variety from the control & Buhans Issues for the variety from the saisins - R07 Henat Sun dried Tayefee (name for the variety from the	AF AF AF AF AF AF		x x		x 6.56 X	x <100			<100											001>				<100
557 260 237 261	807-88-51-11-10-2015-557- 807-88-51-9-10-2015-217-	Ralaine - R67 Kabul Some Roma for the unitaria from the unitaria Ralaine - R67 Kabul Some Roma for the unitaria for the unitaria Ralaine - R67 Kandabar Some for the unitaria from the unitaria Ralaine - R67 Kandabar Some for the unitaria from the unitaria	AF								<100															<100 <100
202 262 229 263	807-6N-52-9-8-2015-202- 807-6N-52-9-9-2015-229-	Salahan - Ratio Kandhar Sanakan - Ratio Sanakan - Ratio Kandhar Sanakan - Ratio Sanakan - Ratio <td>34 34 34</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td><100</td> <td></td> <td><100 <100 <100</td>	34 34 34								<100															<100 <100 <100
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581 267	RD5-KBL-51-11-10-2015-581- RD5-KBL-51-10-10-2015-436-	Ration - Ref. Robut - Institute Advisory Lower for the uniferst from the usual I Rations - Ref. Robut - Robut - Robut - Ration (unification Ration) (unification) (uni	AF								<100															2.21 <100
435 268 9/7/2015 52 269 460 2270	R01-H-54-8-12-2015-052-005 R07-KBL-51-11-10-2015-460	Raisins - R01 Henzt Medium Quality Round Green Raisin ID-field in Hunde and in much Rouse-Himmish Hannal Raisins - R07 Kabul Sun Arived Tayefee (mare for the variety from the porth) & Kebout Insure for the variety from the south) Sun Arived Romani Raisin Lun which Back to color. has a	17		x x		X 6.38 X	X 18.27			<100											1.75				<100 <100 <100
404 271 365 272 400 273	RDS-KBL-S1-10-4-2015-404- RDS-KBL-S1-10-4-2015-365- RDS-KBL-S1-10-10-2015-410-	Raisins - RGS Kabul into an concord arean flavor but small seeds, often exco San dried Shorthi Raisin (uu dried, black in color, bas a stopar ecocord area flavor but small seeds often exco Raisins - RGS Kabul San dried Shorthi Raisin (uu dried, black in color, bas a San dried Shorthi Raisin (uu dried, black in color, bas a									<100															<100
482 274 469 275	R02 KBL-51-11-10-2015-582- R04-98-51-10-28-2015-669-	Rainin - Rots Kabul Sun (Jun Khani) pun king bank hin ing kabula king king king king king king king king	alo Af sin Af								<100 <100															<100
423 276 47 277 26 278	R05 KB-51-10-10-2015-423- R08 KB-52-8-4-2015-047- R06-8-52-8-3-2015-025-	Ratistre - RDS Kabul etensor concent around flavore but small samety offens around Ratistre - RDB Kabul Small red Ratistic or currents (juun dried and stimed in dirt, burstleward reduces in other datase and halost another burstleward reduces in other datase and halost another burstleward reduces in other datases and halost another burstleward reduces in other states from the	A bate			+ 1					<100 <100 <100 <100				<u> </u>					+						<100 342.95 <100
49 279 506 280	R08-9-52-8-4-2015-049- R04-98-51-10-28-2015-506-	Anthree ADB Environ Texamo for the variant from the workshill Balation Texamo for the variant from the workshill Balation Environment (Junn Briefel and Utrime In Inde Section 40 and Utrime Inde Section 40 and 10	AF - AF s in AF						L		<100															12.40 5.24
48 281 448 282	R25-9-53-8-4-2015-048- R25-KBL-51-11-20-2015-648-	Anton - Marine Marine Local E allows terms for the uniter both as control Exhine - Holl Bable Bable Bailton Correction (and devided and devide local Exhine - Holl Parvana) (and the final and correction (and the uniter) Exhine - Holl Parvana) (and the uniter) (and the uniter) (and the uniter) Exhine - Holl Bable Bable Society (and the uniter) (and the uniter) Exhine - Holl Bable Society (and the uniter) (and the uniter) (and the uniter) Exhine - Holl Bable Society (and the uniter) (and the uniter) (and the uniter) Exhine - Holl Bable Society (and the uniter) (and the uniter) (and the uniter) Exhine - Holl Bable Society (and the uniter) (and the uniter) (and the uniter) Exhine - Holl Bable Society (and the uniter) (and the uniter) (and the uniter) Exhine - Holl Bable Society (and the uniter) (and the uniter) (and the uniter) Exhine - Holl Bable Society (and the uniter) (and the uniter) (and the uniter) Exhine - Holl Bable Society (and the uniter) (and the uniter) (and the uniter) Exhine - Holl Bable Society (and the uniter) (and the uniter) (and the uniter) Exhine - Holl Bable Society (and the uniter) (and the uniter) (and the uniter) Exhine - Holl Bable Society (and the uniter) (and the uniter) (and the uniter) Exhine - Holl Bable Society (and the uniter) (and the uniter) (and the uniter) Exhine - Holl Bable Society (and the uniter) (and the uniter) (and the uniter) Exhine - Holl Bable Society (and the uniter) (and the uniter) (and the uniter) Exhine - Holl Bable Society (and the uniter) (and the uniter) (and the uniter) Exhine - Holl Bable Society (and the uniter) (and the uniter) (and the uniter) Exhine - Holl Bable Society (and the uniter) (and the uniter) (and the uniter) Exhine - Holl Bable Society (and the uniter) (and the uniter) (and the uniter) Exhine - Holl Bable Society (and the uniter) (and the uniter) (and the uniter) Exhine - Holl Bable Society (and the uniter) (and the unite	AF AF								<100															11.75 <100
28 283 43 284 258 746	R08-8-52-8-3-2015-028 R08-8-53-8-3-2014-043 R05-KBL-51-10-8-2005-748.	Extent - No. Kindo - Sono anno concert canada meta mais and a senda s	AF AF								<100												+ +			0.44 <lod <lod< td=""></lod<></lod
52 286 264 287	R08-8-53-8-4-2015-052- R06-KRL-55-8-29-2015-264	Raisins - 666 Baltin San Proceeds around the result and schema room of the result and schema room of the result of									<100															2.75
2.64 2.87 570 2.88 4/02 2.89 4.81 2.00 560 2.01 580 2.01	R07 KBL 51-11-10-2015-570- R02-98 51-10-28-2015-470-	Notes Autom concord search frame that restrict conduct when Rainins - R67 Kobul Sain diried Trapier (name for the water) from the control of the scalar (from the control of the scalar (from the scalar) Rainins - R62 Paneais Median Quality (scalar (from the scalar)) Rainins - R62 Paneais Median Quality (scalar (from the scalar)) Raining - R62 Paneais Median Quality (scalar (from the scalar)) Median Quality (scalar (from the scalar)) Median Quality (scalar (from the scalar))	AF ade AF								<100															<100 <100 1.27
481 290 580 291 573 292	nut 99 51-10-28-2015-681- R02-681-51-11-10-2015-580- R02-681-51-11-10-2015-570-	Rations - R62 Parwan Medium Quality Long Green Seedless Rabio (Direct in sh and in much hows: Rishnikh Mixma) Rations - R62 Kabul Medium Quality Long Green Seedless Rabio (Direct in sh and in much hows: Rishnikh Mixma) Rations - R62 Kabul Medium Quality Long Green Seedless Rabio (Direct in sh and in much hows: Rishnikh Mixma) Rations - R62 Kabul Medium Quality Long Green Seedless Rabio (Direct in sh and in much hows: Rishnikh Mixma)	ade								<100 <100 <100								_							<100
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556 295 10/10/2015 225 296	R07-KR-51-11-10-2015-556- R04-KR-51-10-3-15-325-008	Raisins - 807 Kabul Sun drivel Taysfee (name for the variety from the north) & Akissa kanne for the variety from the such) Raisins - 804 Kabul Medium Quarky for Sailing and for Daysfee rice drives and basics could be drive and basic could) Medium Quarky for Sailing for drives and basic Medium Quarky Long Cores Seedless Raisis (Drivel in the Sailing County Sailing Sailing For Seedless Raisis (Drivel in the Sailing County Sailing County Sailing County Sailing	AF	N 34(30)55,3600	x x	$+ \neg$	X 6.72 X	X 9.32			<100									+ - +		12.34				<100 <100 <100
417 299	R02-KBL-51-10-10-15-417-	Raisin - Ruz Kabal and in mut bouns Kishmish Kisanal Raisins - Ruz Hierat Medium Rud bouns Kishmish Kisanal Raisins - Ruz Kishmish Kisanal Raisins - Ruz Kabal Medium Quality Long Green Seedless Raisin (Diried in sha	AF A	N34,20,4,5899 642-11-34,8200	х х		X 6.89 X	X 46.20			<100 <100 <100				_					+ +		<100				<100 <100 <100
20/7/2015 36/9 300 9/7/2015 5.3 301 9/8/2015 1.1 302 9/8/2015 2.0 303	R03 48L-51-10-14-15-369-017 R01 ++ 51-8-12-2015-053-003	Raisin * Nur Raisin * Nur Raisin * 62 Kabul High Quality Poundskhan Raisin Försten Jaho Kabul Raisins - 601 Henst Henst Henst	Kabul, Mandawi AF	N 34(30)55,00 6.60-10-34 3100 N 34(30)2,8999	x x x x		X 7.12 X X 6.98 X	X <100 X 15.01			<100											< LDD Trace				<100 <100
9(8/2015 11 202 9(8/2015 20 203 9(12/2015 47 204	R08+H52-8-9-15-011-001 R08+H53-8-9-15-0220-002 R02+H53-8-11-H5-0007-444	Ration - 600 Henst Enrall med Falsion or currents (un drived and stimet in order for ultravent induce in order and the state model). Raisins - 600 Henst Small med Falsion or currents (un drived and stimet in dir. Incelly used million in frier drives and baland excels). Raisins - 800 Henst Median Quarky (under state) and baland excels). Raisins - 800 Henst Median Quarky (under state) and drived excels).	Haji Husain Store, Herat AF	E2-13-34 5400 N/Av N34,2027,3389 E22-13-05490 N34,20;39,2599 E52-11-27,6700	x x	x		X 15.01 X 8.60 X 8.67 X 7000			<100											 <id0< li=""> <id0< li=""> <id0< li=""> <id0< li=""> <id0< li=""> </id0<></id0<></id0<></id0<></id0<>				10.43 <1.00 <1.00
69 205 9/12/2015 59 206	R02 + 52 - 6 - 12 - 15 - 05 69 R02 + 54 - 10 - 15 - 0569	Excline - Ho2 Henrit Medin - Junity Log Cens Reveal National Annual An	~		x x x		x 6.88 X x 7.28 X	X <100			<100									+ +		<.00				<100
8 208	R01-H-53-8-9-2015-008-	Raisins - R01 Herat Herat	ade Diabad Herat AF	N 34,20;4(83999 662:11:24:5400 N 34;4(49,9500 662:13:24:4999 N 34;20;44,0500	x x		X 728 X X 623 X	X 12.18			<100 <100											9.67				<100 <100
9/13/2015 12 2009 9/12/2015 15 310	801-H-52-8-11-15-012-007 802-H-52-8-12-15-015-005	Raisins - RD1 Henst Middlim Quarty Round Lineo Asian Infract Inducts and Isonatoma Alianobia Manasi Raisins - RD2 Henst Middlim Quarty Long Green Seedless Raisin (Diried in sha and is much house Alianobia Manasi Medium Quarty Round Screen Raisin	ade Jada-e-Hesar, Mandawi, Herat AF	N 34,20;39,3620 662-11-27,4100 N 34,20;39,7299	x x x x x x	\pm	X 7.28 X X 7.22 X X 6.64 X	x 5.00 x 5.00 x 5.52			<100 <100 <100											 <ibb< li=""> <ib< li=""> <ib< li=""> <ib< li=""> <ib< li=""> <ib< li=""> <l< td=""><td></td><td></td><td></td><td><100 <100 <100</td></l<></ib<></ib<></ib<></ib<></ib<></ibb<>				<100 <100 <100
9/7/2015 14 212 4/91 213	R01-95-39-9-9-15-013-006 R01-9-53-0-12-2015-014-002 R06-9R-51-10-28-2015-095-	Names - 401 Heats Operating States and A most development Autors Ration - 801 Heats Medium Quality Round Green Russin Ration - 801 Heats Medium Quality Round Green Russin Ration - 805 Heats Medium Quality Round Green Russin Ration - 806 Parwars Sum dried Ghastri Rakin Juan Kindhan Kin	Jada-e-Hesar, Mandawi, Herat AF Jada-e-Hesar, Mandawi, Herat AF AF	N34;20;39,6199 643-11-27.0899	x x x		X 86.0 X X X X X X X X X X X X X X X X X X X	X 5.52 X 24.79			<100 <100 <100											<001 < 100				<100
20/7/2015 344 314 352 315	R03 KBL-51-6 10 15-346-022 R05 KBL-51-10 + 2015-352	Mills Mills Product and the second and	Kabul, Mandawi AF	N 34;30;25,0200 669-#-35 35 355	x x		X 6.72 X	X <100			<100											5.19				<100
242 216 496 217 479 310	R06-98-51-0-2015-242- R06-98-51-10-28-2015-496- R06-98-51-10-28-2015-496-	Balaine - 650 Kandahar Fanal m 6 Balaine or currents (un divide and stime) in dirit. Incally used mixing in indirection and baland ascelul San Stried Chaussi Rakin (un direct balanch in color, has a string concerd mass filters balanch or color, has a string concerd mass filters balanch or the Davide - 600. Davide - 600. Parware San Stried Chaussi Rakin (und balanch in color, San Stried Chaussi Rakin (und balanch in-curit) San Stried Chaussi Rakin (und balanch in-curit)	AF AF AF			\pm					<100 <100 <100 <100															11.89 <1.00 <1.00
4/9 218		Raisins - RDG Parwan Sun pred unaam kaun juur dred, back in coor, bas a strong concord grade fizzor but small seeds often	*			1			1	L	<100								I		I					<100

518 219 R04-PR-51-10-29-2015-518- Raisins - R04												 					 	
53 220 R09-9-52-6-4-2015-053- Raixin R08 265 221 R06-KB-55-8-20-2015-265- Raixin R06	Parwan Balkh	Medium Quality Red Rainin (um dried locally used raintes in rice dishes and hained secold) Famili red Rainin or currents (um dried and strived in dirt, localit used minim in rice dishes and hained secold) San dried Grauss Rainin (um dried) black in color, has a strong concernisme filtwork bat small seeds often site. Duality concernisme filtwork bat small seeds often site. Duality concernisme filtwork bat small seeds.		N N									<100 <100 <100					
Digitalization Status National Activation of the property of the prop	Kabul	IGolden-Hish Value Dried in shade and in mud house- Medium Quality Long Green Seedless Rakin (Dried in shade	abul, Mandawi	AF N34;30;54,2799 E69:10:35.0500 M34;20;39,2599	x	x	x	7.35	x		X 5.00 X 18.80		<100					
510 324 R04-98-51-10-28-2015-510 Railins - R04 30/7/2005 333 325 R03-48L-51-10-3-15-333-012 Railins - R03	Parwan	Medium Quality Red Raisin (sun dried locally used raisins in rice dishes and baked ecods) liteh Quality Stundarishari Raisin		AF 11427.6700 AF N34.20;55,8099 E00-10-25.9200	×	x	x	7.71	×		X <100		<100					
10/10/2015 281 227 R04-K8L-51-6-10-15-381-010 Raisins - 804	Parwan Kabul	Medium Quality Red Raisin (sun dried locally used raisins in rice dishes and baked ecode)		AF N.34;30;54,3200	×	x	x	6.65	x		X 8.21		<100					
565 328 R03-KBL-51-11-10-2015-565- Rateline - R02 30/7/2015 360 329 R03-KBL-51-10-4-2015-360-029 Rateline - R03	Kabul	and in multiplease kickwich Khanal High Quality Shundurishani Raisin	abul, Mandawi	AF N34(30)55,2899 AF Lat: 34,30543299 AF Lat: 34,30543299	x	x	x	7.07			X <100 X <100		<100 <100 <100					
Lag (2) Linity Linity <thlinity< th=""> <thlinity< th=""> <thlinity< t<="" td=""><td>Kabul</td><td>IGolden-Jilde Value Picela in shorte and in mut horoze. Sun dried Shornali Rahini Jun dried, black in color, has a stone corecord areas Rayor but small seeds, often exported Sun dried Shornali Rahini Jun dried, black in color, has a stone corecord areas Rayor but small seeds, often exported Medium Quality Rie Rahini Jun dried locally used nahins in</td><td></td><td>Long 69.103464000</td><td></td><td></td><td>*</td><td>0.49</td><td>-</td><td></td><td>×</td><td></td><td><100</td><td></td><td></td><td></td><td></td><td></td></thlinity<></thlinity<></thlinity<>	Kabul	IGolden-Jilde Value Picela in shorte and in mut horoze. Sun dried Shornali Rahini Jun dried, black in color, has a stone corecord areas Rayor but small seeds, often exported Sun dried Shornali Rahini Jun dried, black in color, has a stone corecord areas Rayor but small seeds, often exported Medium Quality Rie Rahini Jun dried locally used nahins in		Long 69.103464000			*	0.49	-		×		<100					
	Kabul Kandahar	rice dishes and baked reads) High Quality Shundurishani Ralain ka Golden-sileh Valas Dried in shade and in mud house- Medium Quality Round Green Ralain Disket in shade and in mud house-Kishmish Khana) Redium Quality Round Green Ralain Kedium Quality Round Kedium	abul - Mandawi unjwaee dist. Kandahar	AF 104/30/55.6499 E09-10-26.0400 AF 102:34/25,27994 E05-48-50.000 AF 104/25/54.E100 E09-10-34.9599	x x	x	x x	7.14	x	_	X <lod X 14.97</lod 		<100					
474 336 R06-PR-51-10-28-2015-474- Rainins - R06	Kabul Parwan	Medium Quality Round Green Railin IDried in shade and in mud house-Kishmik Manas) San dhied Chaani Railin (sun dhied, black in celor, hara attrong concord mane Rison but small seeds, often Medium Quality Round Green Railin		AF N34/30/54/6100 E69-10:34.9599 AF N34/30/54/4600 AF N34/30/54/4600	×	x	x	6.28	×		X <100		<100					
10/13/2015 454 338 R02-KBL-51-10-11-2015-454-013 Raisins - R02	Kabul	Medium Quality Long Green Seedless Raisin (Dried in shade Ka		AF 100,10,25,000 AF 134,20,55,259 AF 134,26,55,259 AF 134,26,55,259 AF 134,26,25,260 AF 134,26,26,00	x x x	x x x	x x x	6.34 6.66 7.3	x		X <100 X <100 X 14.03		<100 <100 <100					
§713/2005 225 340 826-46-53-9-61-54225-0012 Rainin-1601 431 341 826-48-54-20-5205-433 Rainin-165 10/10/2005 342 342 86-482-52-20-55-432-066 Rainin-166	Kabul Kabul	In fact in shock and in much house. Einheide Weard		AF.	×	x	x	6.85	x		X 7.83		<100					
20/6/2015 280 342 R01-KBL-51-4-10-15-280-016 Raisins - 801	Kabul Parwan	mention quartity field and another mention durating the strength durating the strength duration mention quartity field another Medium Quartity Faceus Coreen Rasilin Medium Quartity Faceus Coreen Rasilin Medium Quartity Face Rasining turn direct Docation Medium Quartity Medium	abul, Mandawi	AF N34(30)54,4600 660-10-35 1400	×	x	x	6.43	x		X 8.93		<100					
233 246 809.481-51-10.4.2015-225. 0.41-10.00	No.	Other DD Mined Builds		AF N31;34;27,660 165:48:47,190	×	x	x	6.36	×		X 12.66		<100					
10/10/2015 285 247 804-KBL-S1-05-515-205-012 Rainin: - R04 30/7/2015 201 248 R03-KN-5L-98-5L-2015-005 Rainin: - R03	Kabul Kandahar	Medium On Hotel Adams Medium Quality Red Rakin (sun dried locally used rakins in Ka Kigh Quality Shundurshani Rakin Kigh Quality Shundurshani Rakin Kighten-Hish Value Dried in shade and in mud boyae-	abul, Shahr e-Naw and dist. Kandahar	AF N34:30;54,4600 E69-10:34:5299 AF N21;34;27,729 E65-48-47:829	x	x	x x	6.75	x		X 5.90 X <lod< td=""><td></td><td><100</td><td></td><td></td><td></td><td></td><td></td></lod<>		<100					
10/10/2015 286 250 R04-KBL-51-4-10-15-386-011 RainimR04	Kabul	Medium Quality Red Raisin Jsun dried locally used raisins in			×	x	x	6.92	x		x 7.92		<100					
23	Kabul	Medium Quality Round Green Raisin (Driad in shade and in mort broase all shmish Khana)	abul, Mandawi abul, Mandawi	AF N34/30/54,4620 F09-10-25,1000 AF N34/30/55,51200 AF N34/30/55,51200 AF N34/30/55,51200	x	x	x	6.85	x		X <100 X <100		<l00 <l00 <l00< td=""><td></td><td></td><td></td><td></td><td></td></l00<></l00 </l00 					
		Sun dried Shomali Raisin (sun dried, black in color, has a stone concerned aroue Raise had small sands often associated High Quality Shundurkhani Raisin Isa	abul - Mandawi	AF N34(30)55,5399	x	x	x	6.34	x		X 8.17		<100					
10/12/2015 458 256 R04-K8L-51-10-11-15-458-007 Rainins - 804	Kabul	Medium Quality Red Raisin (sun dried locally used raisins in rice drifter and baked acods) Medium Quality Red Raisin Isun dried locally used raisins in	abul, Mandawi abul - Mandawi	AF N34(30)55,5399 E03-10-25,7200 AF N34(30)55,2899 E03-10-25,4200 AF N34(20)55,2899 E03-10-25,6799	x x	x	x x	7.01	x		X <lod X <lod< td=""><td></td><td><100</td><td></td><td></td><td></td><td></td><td></td></lod<></lod 		<100					
228 257 801-484-51-10-3-205-328- Raisins - 801 426 258 805-484-51-10-10-2015-426- Raisins - 805	Kabul	Modum Quarty second uneer kaun (Dised in shade and in much bease Kähnish Khana) Sun dried Shomali Raisin (sun dried, black in color, has a strong concord grace Rayor but small seeds, often exported		AF	$\pm \pm$	$\pm \pm$			$\pm \pm$	+ =			400/400					
400 269 R05-KBL-51-10-10-2015-420- Rasins - 825 917(7055 261 261 R03-KBL-51-10-10-2015-420- 917(7055 261 261 R03-KBL-51-10-10-2015-420- Rusins - 825	Kabul	San dried Shomali Raisin Juun dried, black in color, has a shonar executed arous fluore but small sands: often associated High Quality Shundarishani Raisin	abul, Mandawi	AF N34(30)55,5800	×	x	x	6.65	x	++	X <100		<100 <100 <100					
401 263 R02-KBL-51-10-10-2015-401- Raisins - R02	Kabul	rive dishes and balant accords Medium Quality Long Green Seedless Rakin (Dried in shade		AF 100-10-33 0000	\square		-						<100					
461 364 807-488-51-11-0-3015-463- Raisine - 807 343 365 865-68-52-410-15-243- Raisine - 805	Kabul Kandahar	Suc dried Tappfee (name for the variety from the north) & abieus insme for the unriety form the numb) Suc dried Shomali Rabin (num dried, black in color, has a stonar record aroun flauer har a wall such rolen, has a Suc dried Shomali Rabin (num dried, black in color, has a		N N									<l00< td=""><td></td><td></td><td></td><td></td><td></td></l00<>					
262 267 R05-K8L-3A-8-25-15-262-002 Raisins - R05	Kabul	Sun dried Shomali Raisin (sun dried, black in color, has a strong concord grace flavor but small seeds, often esported		24 24 24	\models				+			 	<100			_		
10/13/2015 389 370 R05-K8L-51-4-10-2015-389-025 Raisirs - R05	Kandahar Kabul	Died in shade and in mud house-Kishmish Khana) Sun dried Shomali Raisin (sun dried, black in color, has a	horandam Market, Kandahar abul, Mandawi	AF N31;34;25,389 E65:48:50.140 AF N34;26;54,6100 E69:10:24.9599	x	x	x x	6.32	x		X 15.90 X 6.59		<100					
411 371 803-686-58-100-3015-611- Railow-802 30/7/2005 216 372 803-686-58-98-15-256-002 Railow-803	Kabul Kandahar	and in multihouse Kishmish Khanal	ingbesar dist. Kandahar	AF N31;34;25,5700 E65:48:50.000	x	x	x	6.85	×		X 5.97		<100					
405 375 R05-KBL-51-10-10-2015-405- Raising - 805	Kabul	sun dried shomas kalein jilun dried, diack in color, has a	abul, Mandawi	AF N34;30;53,9199 AF F00-10-25 3000 AF	×	x	x	6.69	×		x 5.91		<100 <100 400/400					
Add Add <td>Kandahar</td> <td>intervent networks arount factor but sensil states rollers asymptotic Small red Raisin or currents (sun dried and stirred in dirt, Increalized relative in rise datum and haised anode) Madian Challes Back Raisin from direk (scrib) under raising in</td> <td></td> <td>N N</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td><100</td> <td></td> <td></td> <td></td> <td></td> <td></td>	Kandahar	intervent networks arount factor but sensil states rollers asymptotic Small red Raisin or currents (sun dried and stirred in dirt, Increalized relative in rise datum and haised anode) Madian Challes Back Raisin from direk (scrib) under raising in		N N									<100					
20/7/2005 284 379 R03-K8L-51-10-6-2005-386-020 Raisins - R03		Small red Raisin or currents (sun dried and stored in dirt, locally used raisins in rice dishes and baked escelul High Quality Sundarkhane Raisin IGolden-Hieh Valar Dried in shade and in mud house-	abul, Mandawi	AF N.H.30;54,4600 669:10:34,7500	x	x	x	6.29	x		Х <100		<100					
509 360 Rd+99.51-10.28.2015-529 Raisin-864 30/072051 251 381 Rd+48.53-410.15.28.2021 Raisin-801 10/02051 281 382 Rd+48.43-410.15.281.4021 Raisin-804	Kabul	Medium Quality Round Union Kasan (Dried in shade and in much house Kishmish Khana) Medium Quality and in much house Kishmish Khana)		AF N.H.20;55,8999 E0:10:23.4200 AF N.H.20;54,5000	x	x	x	6.45	x		X <lod X 7.68</lod 		<100 <100 <100					
32/7/2015 324 383 R03-K8L-51-05-3-15-224-007 Raisins-R03 32/6/2015 368 384 R05-K8L-51-4-10-15-368-019 Raisins-R01	Kabul	rice dense and based rocali High Quality Shundurishani Raisin Ka	abul - Mandawi abul, Mandawi	AF N34(20)55,9999 E99-1023,4200 AF N34(20)54,8000 E99-1023,8200 E99-1023,8209 AF N34(20)55,3300 E99-1023,6599 AF N34(20)54,8000 E99-1024,5590 AF N34(20)54,8600 E99-1024,5500	x	x	x x	6.25	x		X 17.14 X Trace		<100					
20/7/2015 229 286 R03-K8L-51-10-3-15-329-014 Raisins - R03	Kabul		abul, Mandawi	AF N 44(40)56(0800	x	x	x	6.28	x		X 10.79 X <100		<100					
50 347 R68-51.8-4.2015.555 Rainin - 168 511 388 R04-39.51.10.28-2015.511 Rainin - 168 512 389 R04-39.51.10.28-2015.512 Rainin - 164	Parwan	Invaliduated relative in rise dealers and hadred enough Medium Quality Red Ration (sum dired locally used rations in rise dealers and hadred enough Medium Quality Red Ration (sum dired locally used rations in		AS AS									<100 <100 <100					
33/7/2015 353 300 Rd3-KBL-51-4-10-15-253-021 Rainins - Rd3 10/12/2015 434 391 Rd6-KBL-51-0-10-15-434-023 Rainins - Rd6	Kabul Kabul	rine dahasi and hakari anooli High Quality Shundiardana Ralah Gostana Jakar Mundiardana Ralah Sun dried Ghami Ralah Juan dried, black in color, has a strone concered mane fixero bat muli seeds, often Medium Quality Round Graun Balah	abul, Mandawi abul, Mandawi	AF N34(30)55,8999 E09-10-32.4330 AF N34(30)53,6399 E09-12-34.9900 	x	x	x x	6.66	x		X <lod X <lod< td=""><td></td><td><100</td><td></td><td></td><td></td><td></td><td></td></lod<></lod 		<100					
	Kabul Parwan	(Dried in shade and in multiplace, Kishmish Khana)	ibul, Mandawi	A5 609-10-32 2999 A5	×	x	x	6.43	×		X <100		<100					
440 204 R55-KRL-52-00-10-2015-640- Rainer-R65 20/7/2005 216 395 R04-KRL-52-00-31-5-305-011 Rainer-R03 567 206 R02-KRL-52-10-315-567- Rainer-R02	Kabul Kabul		abul - Mandawi	AF N34(30)55,5309 AF 609-10-35,9700 AF	×	x	x	7.07	x		X <100		<100 <100 <100					
464 207 R06-99-51-10-28-2015-454 Rasins - R06 559 208 R02-481-51-11-10-2015-558- Rasins - R02	Parwan	Medium Quality Long Green Seedless Rakkin (Dried in stude and in mult house-Köhnish Khana) San dried Ghaani Rakin (sun died, black in color, Assa a strong control grang filosophile small south often Medium Quality Long Green Seedless Rakin (Dried in stude mol in met house) filotimish Hana)		NS									<100					
343 209 801-482-51-10-6-2005-343- Rainine - 801 372 400 805-482-51-10-6-2005-372- Rainine - 805	Kabul	and is much because Arthonish Rhanosh Medisam Quality Rocand Genera Rasian I'r ford in sharder and is much because Arthonish Rhanosh San dhried Dhannali Russian Qual dried, Statick in color, has a stranser non-mod armon Russer, but a small sander, orden a senontant Fan abried Rocan Russer, but a small sander, orden a senontant		AF									<100					
10/10/2015 284 401 R66-KRL-51-05-51-284-005 Ration - R66 8/11/2015 212 402 R03-KH-52-9-015-2012-001 Ration - R03 220 402 R08-KH-52-9-015-2012-001 Ration - R03	Kandahar Kandahar	High Quality Shundurkhani Raisin Kolden Jileh Value Dried in shafe and in mud house.	horandam Market, Kandahar	AF N34/30/54/4600 E00-10-34/0000 AF N32/34/36/759 E00-40-50/340 AF	x	x	x	6.61	x		X 9.91 X 12.77		<100 <100 <100					
463 404 803-98-51-55-28-15-463- Raine - 802	Parwan	Medium Quality Long Green Seedless Rakin (Dried in shade and in mud house-Kishmish Khana) Medium Quality Red Rakin (sun dried locally used rakins in rice dishes and baked acode)	abul, Mandawi	AF N34:30;57,8399 669:10:32.6199	x	x	x	7.17	x		X <100		<100					
10/13/2015 419 406 805-K8L-53-10-10-35-419-025 Raisins - R06 10/13/2015 422 407 805-K8L-53-10-15-432-626 Raisins - R06	Kabul	has a strong concord grapp flavor but small useds, often Sun dried Ghazni Ralsin (sun dried, black in color, has a strong concord grapp flavor but small useds, often	abul, Mandawi abul, Mandawi	AF N34/30/57/3399 (19)-10:22.6199 AF N34/30/57/3399 (19)-10:22.6199 AF N34/30/54,2100 (19)-10:25.0320 AF N34/30/53,200 AF N34/20:39,2599 AF N34/20:39,2599	x x x	x x x	x x x		x x x		X <100 X <100 X <100		<100					
48(2)255 66 400 823:454.8-13:50005600 knows-402 9/7.0216 7.1 400 851.64.12:2015.0072.004 Mains-402 10/20205 250 410 84.64.51:40.15:2015.0072.004 Mains-402 10/20205 250 410 84.64.51:40.15:207.005 Mains-402 10/20205 257 421 81.64.61:10:15:27:055 Mains-402			labad. Herat	AF N34;14;50,1999	x	x	x	6.28 6.93 6.69	x		X <100 X 10.53 X 7.63		<100 <100 <100					
9/7/2015 9 412 R01-H-54-8-12-2015-009-001 Raisins - R01	Herat	Medium Quality Kound Linein Kalish (Didad in shade and in must becau. Eishmish Khana)	abul, Mandawi ida-e-Hexar, Mandawi, Herat	AF N34(30)57/0899 E03-10-32 2000 AF N34(20)3009 E43-11-27 0200	x	x	x	7.11 7.21	x		X <100 X 5.00		<100					
	Kabul Kabul Kandahar	(Delect in shade and in much broase Alabertah Wasna) Net Medium Quality Round Green Raisin (Delect in shade and in much broase Alabertah Wasna) Ka	abul, Mandawi	AF N34/30/54/6100 F00-10-34/0000 AF N34/30/55/8999 F00-10-33/4200 AF	x	x	x x	6.48	x	+	X <100 X 15.12		<100 (100 (100 (100 (100 (100 (100 (100					
364 416 809-K8L-SL-10-8-2015-364- Raikins - 809 221 417 801-80-52-96-2015-221- Raikins - 801	Kabul Kandahar	Other OR Mixed Raisin Medium Quality Round Green Raisin Dide in whole and in multi-research internal		и и и					+				<100 <100 <100					
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10/28/2015 456 604 93 605	PD VBL 51-10-11-546-605 Plexibio-145 Khall Excel Telephone half with purple approximation PO3-50-30-31-2015-033- Plexibio-1450 Camargination Plexibio-1450 Camargination PO3-50-30-31-2015-033- Plexibio-1450 Camargination Plexibio-1450 Camargination PO3-50-30-31-2015-034- Plexibio-1450 Camargination Plexibio-1450 Camargination PO3-50-30-31-2015-034- Plexibio-1450 Camargination Camargination Camargination PO3-50-31-2015-034- Plexibio-1450 Camargination Camargination Camargination PO3-50-31-31-31-31-31-31-31-31-31-31-31-31-31-	Kabul, Mandawi Af	Lat: 34,30551799 X	x	x	7.37 X	115.56 12.17 570.84	_	13.13 576.88	1189.13 1120.50	c100					_	<100
10/28/2015 445 606 10/28/2015 432 607	P01-K8L-S1-10-11-15-465-003 Pstachio-P01 Kabal Kook Pipachio-(ppm shell with purple purper with) P01-K8L-S1-10-20-5F-832-004 Pitashio-P01 Kabal Kook Pitachios (ppm shell with purple	Kabul, Mandawi AF		x	x	7.86 X 7.53 X	18.12 8.61		6.25	4.93	c100 c100 001						<100
349 608	Part 61: 40: 50: 50: 400 Parts Par	AS	N36/42/5,0400	x			<100		< 100	0.48	<000						<100
11/17/2015 58 610	P04-9-3-8-3-15-989-001 Pittschio-P04 MARG Othersafelist of Patachios P015-51-16-30-2015-558-015 Pittschio-P01 Samangan Konki Pittschios (open shifi with purple open address open address o	Safi dry fruit market, Mazar AF AF	N 26:40:50400 X 667:6:48:01999 X	x	x	7.71 X	<100 <100		19.88	<lod< td=""><td><100</td><td></td><td></td><td></td><td></td><td></td><td><100</td></lod<>	<100						<100
11/17/2015 76 611 11/17/2015 67 612	P04-8-55-8-4-2015-075-020 Pistachio - P04 Balleh Other varieties of Pistachios P015-551-80-30-2015-067-016 Pistachio - P01 Samagan Protection Adv	LT AF		x x	x	7.4 X	<100 <100		<100 28.75	<lod 141.66</lod 	<000						<100
212 613 11/17/2016 79 614	P03+R8L-S1-IB-3-2015-313- Pistschio - P03 Kabal Whandish-e-util@Pistschios(strong Tavor and writely what? P01-551-30-31-2015-079-017 Pistschio - P05 Samangan Knok Pistschios (open shell with purple	A5 A5		x	x	7.81 X	10.90 < 100		< 100	0.65	c100						2.55
96 615	P02-5-51-10-21-2015-096- Pittachio - P02 Samangan Punktura Pittachios (closed shell with purple public dkini P02-5-51-10-21-2015-096- public dkini P02-5-51-10-21-2015-096- Pittachio - P02 Samangan Punktura Pittachios (closed shell with purple	AF AF					Tace		Trace	<100	<100 Contract Contrac						<100
80 617	Pacenta series unarear in annual pacenti de la contraction en la contraction de la c	AF			x		<100		27.13	2.72							
10/28/2015 18 618 10/28/2015 421 619	P01-8-53-8-3-15-038-000 Pittschio - P01 Balte Korak rutacher (open timel with purple P01-828-51-10-30-15-431-008 Pittschio - P01 Kabul Korak Pittschio (open shell with purple p01-628-51-10-30-15-431-008 Pittschio - P01 Kabul Loder Kini (AF Kabul, Mandawi AF		x			8.23 <100 <100		6.25	0.36 <lod< td=""><td>(00) (00)</td><td></td><td></td><td></td><td></td><td></td><td><100</td></lod<>	(00) (00)						<100
10/28/2015 293 620 10/28/2015 17 621	No. 6, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0,	Kabul, Mandawi AF	Lat: 34,30544630 X Long: 69,10345299 Lat: 34,2053999 X	x	х	6.93 X	239.36 10.50			1880.20	<100 Contraction C						<100 <100 <100
10/28/2015 439 622	PO1-K8L-51-10-30-15-439-0011 Pittachio - PO1 Kabul Konk Pittachio (open shell with purple zostarckini)		Lat: 34,2052999 X Loss: 54,114982999 X Lat: 34,26541299 X Loss: 59,10251400	x	x	6.96 X	184.41 <100 <100		< 100	0.81	<100						<100
9 624 11/17/2015 89 624	P044-0-4-0-2-003-09- Prestrike - P04 Maketine - P04 Maketine - P04 Maketine - P04 Maketine - P04-0-561-0-02-009-0-040 Maketine - P05 Samangan - P04-0-040 Maketine - P04 Ma	AF AF	×	x	x	7.88 X	<100 100.18		94.63	14.19	<100						<100
10/28/2015 403 625 74 626	Byte Add to B of B	Kabul, Mandawi AF AF		x	x	7.04 X	200.09 Trace		6.25	1.66	<100						<100
11/17/2015 148 627 9/5/2015 75 628	P015-51-11-2-2015-148-014 Pittschio - P01 Samangan Korak Pittschios (open shell with purple more disk P04-0-54-0-15-075-002 Pittschio - P04 Balteh Other underlies of Pittschios	AF Haji Murad Dry fruit Market, Mazar AF	×	x x	x	7.72 X 7.71 X	<100 30.97 77.28		82.00	82.28	(100 C C C C C C C C C C C C C C C C C C						<100
11/17/2016 88 629	P01-5-51-10-21-2015-088-018 Pistachio - P01 Samangan Korak Pistachios (open shell with purple outer skin) - P01 Samangan Korak Pistachios (open shell with purple outer skin) - Korak Pistachios (open shell with purple Korak Pistachios (open shell with purple)	AF Herat - Ghafood Market AF	The called w	х	x	7.45 X	<1.00 Trace 129.41		Trace	<100	<100						<100
11/17/2015 572 631	Polerka SL-11-16-2015-572-019 Pittachio - POL Kabul zuder dish	AS	x	x	x	2.43 X	<100			<100	<.00						<100
10/28/2015 8 632 UNL 1 633	P02-8-52-8-3-2015-008-002 Pistachio - P02 Balleh PURDATA VIIISTONI (Solida Inite Weth purple outer Kild P02-KIR-S2-11-23-2015-006- Pistachio - P02 Kabal outer Kild Outer Kild	AF AF	Long: 67.64676000 X	x	x	7.55 X X	23.68 27.37 34.77		21.75 25.50	13.57 2942.44	<100						<100
UNL 14 634 UNL 19 635	P02+K8L-S1-11-22-2015-034 Pistschio - P02 Kabul Pubdrar Pittschios (closed shell with purple p02+K8L-S1-11-22-2015-039 Pistschio - P03 Kabul Khandar-+-safd Pittschios (strong flavor and	AF AF		_			6.58		<100	5.74	<100 Contraction C						<100
UNL 6 636	P02+K8L-S2-11-21-2015-006- P02+K8L-S2-11-21-2015-006- P02-K8L-S2-11-2015-006- P02-K8L-S2-11-2015-006- P02-K8L-S2-11-2015-006- P02-K8L-S2-11-2015-006- P02-K8L-S2-11-2015-006- P02-K8L-S2-11-2015-006- P02-K8L-S2-11-2015-006- P02-K8L-S2-11-2015-006- P02-K8L-S2-11-2015-006- P02-K8L-S2-11-2015-006- P02-K8L-S2-11-2015-006- P02-K8L-S2-11-2015-006- P02-K8L-S2-11-2015- P02	N				x	Trace		6.25	1.17	<100						<100
UNL 25 638	Norther of a data of a data Norther of data Norther of a data Norther of data Norther	N N				x	6.48 12.95		< LOD Trace	<lod 0.92</lod 	<100						<100 <100 <100
UNL 8 629 UNL 28 640	P64-KBL-S2-13-22-3015-2081 Pittachio-P64 Kabul Other varieties of Pittachios P03-KBL-S2-13-22-3015-2081 Pittachio-P63 Kabul Mandma-wated Pittachios (strong Tavor and window)	AF AF				x x	<100		< LDD 1071.88	\$1.81 <lod< td=""><td><100 (00) (10) (10) (10) (10) (10) (10) (</td><td><u> </u></td><td></td><td></td><td></td><td></td><td><100</td></lod<>	<100 (00) (10) (10) (10) (10) (10) (10) (<u> </u>					<100
UNL 16 641 UNL 24 642	PSI db, 51: 32 2005 cb; Pathins: PBI DBM Objective ved Physical Stations (Simple Review et al. Stations) PSI db, 61: 12 2005 cb; Pathins: PBI Ball Debutes ved Physical Stations (Simple Review et al. Stations) PSI db, 61: 12 2005 cb; Pathins: PBI Ball Debutes ved Physical Stations) Debutes ved Physical Stations) PSI db, 61: 12 2005 cb; Pathins: PBI Ball Debutes ved Physical Stations) Debutes ved Physical Stations) PSI db, 61: 12 2005 cb; Pathins: PBI Ball Debutes ved Physical Stations) Debutes ved Physical Stations)	A5 A5				×	< 100		Trace Trace	<lod 4.30</lod 	<100						<100
UNL 23 643	P03+682-51-11-22-2015-623- P0aceho - P63 Kabul Whandassafe Potachios (strong flavor and wrinkly chell)				1	×	39.62		43.50	<100	<100					+ +	<100
UNL 3 644 UNL 7 645	rvorves-sa-si-2i-AUU-500- Pitachio-P04 Kabal Othernanistics of Pitachios P04-K8L-52-132-3015-007- Pitachio-P04 Kabal Othernanistics of Pitachios	A5 A5				x	<100		< LOD Trace	0.86 <lod< td=""><td><100</td><td></td><td></td><td></td><td></td><td></td><td><100</td></lod<>	<100						<100
UNL 2 646 UNL 15 647	Name Constraint Constraint <td>AF AF AF</td> <td></td> <td></td> <td></td> <td>x X</td> <td>Trace <100</td> <td></td> <td><100 6.25</td> <td>2.43 <100</td> <td><100</td> <td><u> </u></td> <td></td> <td></td> <td></td> <td></td> <td><100</td>	AF AF AF				x X	Trace <100		<100 6.25	2.43 <100	<100	<u> </u>					<100
9/29/2015 29 24.A 9/29/2015 71 24.9	WD7-H954-8-15-029-004 Floar - WD7 Herat Two mass balances in each regions (Dther det or cement that WD22-H52-8-4-15-071-003 Floar - W12 Herat Type mass balances in that	Herat - AF Herat - Airport road ~~		x	x x	6.56 X X 7.40 X X	X X 10.57 X X <100				<lod 735.00<="" td=""><td>149.05 Trace</td><td></td><td></td><td></td><td></td><td></td></lod>	149.05 Trace					
9/21/2015 301 IBN31/#U210	Automatical of Affairs Option (Nhine often Automatical of Affairs Option (Nhine often Automatical of Affairs Option (Nhine often Automatical often Automatical often Automatical often Automatical Automatica	Herzt - Arport road IX Balkh, Nawabad, Dehdadi AF Balkh, Dry fruit Market AF	N34(20)39,2599 X EX3-11-27 6200 N35(6)27,2500 EX2-213-4200 N35(0)2,2500 X	x x x		7.31 X X	X X <lod 145.01</lod 					100.35			Tas		
10/28/2015 19	Bit Cold S - 50 Col	AF AF	Intelligence X Lat: 36,6127300 X Lat: 36,6217300 X Lat: 36,6217300 X Lat: 36,6217300 X Lat: 36,722303 X	х	x	7.09 X	15.10										
10/28/2015 76 10/28/2015 77	P02+H-S3-8-11-IS-007-001 Pittachio - P02 Herat Muldara Pittachio (scient dell'arith purple page radio) P01-H-S6-8-11-IS-007-013 Pittachio - P01 Herat Koak Pittachio (open shell with purple page radio)	Herat - AF	Long: 62.11851999 Long: 62.11851999 Long: 67.526799	x	x	6.88 X 7.17 X	13.41 72.47										
209 I-N32/90AU 9/28/2005 57 I-N82/69A	W09-80-53-9-15-203- Kandahar Pakistan flour W08-9-53-8-1-55-057-002 Flour- W08 Balish Two – four flour-millen is each of the three regions	PK Balih, Rasoli Co. UZ & AF	Loar: 67.526799 N36;41;59,8200 X E67:5:55.92999 X	x	x	7.12 X X	X X Trace		<u> </u>	-	Тлая	Trace	\vdash		11.51	+ $+$	
56 N	WN95-5-52-16-38-2015-056- Walnut WN95 Samangan Kaghasi Walnuts (paper shelled)	AS Not Market	Lat: 34,30551799Lorg: 69,1 X				85.5										
10/17/2015 1	응전5 5-13 13 33 2012 655 전 88 mbr 9805 Sinnargan Raghast 200 mbr 30 gene nohed 지당 (RL 54 13 15 45 65 66 78 mbr 47 mbr	kalo - Af	N/Av X	x	x	7.45 X	<100										
2	WEG 49: 51-16-28-55 001 039 Fibur - WEI Samangan Asalab Nor Sourd on Nyalao Unijio	Samangan, Zir Dara-e-Zendan AF Herat, Eid gah AF	N(Av X N(A) X CER 00233 X N(A) (252381 CC CC3 148663 X N(A) (352399 X CC3 148663 X N(A) (352399) X CC3 148653 X N(A) (352399) X CC3 148653 X	x	x		X X <1.00				Trace	< 100			< 100		
4	WIGE HIGT - 9.615.502 Wheat - Wood Heart Two warehouses or strange facilities in Mazar-Dourf, Field and Strange facilities in Mazar-Dourf, Field and Strange facilities in Mazar-Dourf, Historia - 1000 825-KIL - 9.25 - 5000 Rainin - 865 Kabal Samithe - 300 Maran - 300 Maran Historia - 2000 Maran - 300 Maran WIGH - 52-9-8-15-000- Floar - WHI Heart Next - 100 Maran	ed Kabul - Shamali AF Herat, Eid gah st. KZ	N/Av X	x	x	6.45 X	X Trace	_							8.34		
9/12/2015 6 8/20/2015 6 9/8/2015 8	Bits Data 2000 Filter Linearge Linearge <thlinearge< th=""> <thlinearge< th=""> <thlin< td=""><td>Herat AF</td><td>Lat: 344,203115999 X Loss: 62,312257000 X N36,02,2599 X 5425,647,200 X N34,227,64,0500 X</td><td>x</td><td>x</td><td>6.5 X</td><td>X Trace 17.11</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td>< 100</td><td></td><td></td></thlin<></thlinearge<></thlinearge<>	Herat AF	Lat: 344,203115999 X Loss: 62,312257000 X N36,02,2599 X 5425,647,200 X N34,227,64,0500 X	x	x	6.5 X	X Trace 17.11								< 100		
9/8/2015 8	R02-H 53-8-2-15-008-001 Raisins - R02 Henst Medium Quality Long Green Seedless Rakin (Dried in shad and in multiple and a series of the serie	P lada e Hesar, Mandawi, Herat AF	647.647.700 A N34,25,44,0500 X 642-11:17.5499	x	X X	7.32 X	X 25.99								<100		
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55	W08-9-52-4-8-15-055- Wheat - W08 Balkh Two - four flour millers in each of the three regions	Rathy, Rasoli Co. AF Horat - Azmi Market AF	X X X X X X X X X X X X X X X X X X X	x	x	7.56 X	<100	-		+		+	\vdash			+	
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64	ALL-V-5-23-811-55-066-012 Altinoid - AD2 Samangan Stokeshall Hard-Abet Altinoids WDB-H-52-8-11-55-064- Wheat - WDB Heat Two – four flour millers in each of the three regions	AF Herat Slo AF	Lut: 26,15739 X Inter. 620(3734) X NA_200905000 ICL:11373700 ICL:1373701 NA_200905000 ED:11373701 NA_200950500 ED:11373701 NA_200950500 ED:11373701	x	x	7.18 X	8.49										
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90 92	WIDS #-33-3-8-5500c Wheat - WDI Basis Anishes MII Fixer of Afgins Offset offset WIDS #-33-3-8-5500c Wheat - WDI Head Anishes and efficience of the set offset WIDS #-53-12-8-15-002c Wheat - WDI Head Anishes first proceed Crap Storage (PKS) bags	Balih, Sherabad Dehdad AF Herat, Zindajan N/A	N 36;40;32,3399 642-2 10 3900 N 34;20;428 642-186402					-		+						+	
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11/11/2015 542 11/14/2015 552	W11-96-31-87-26-15-562-002 Roar - W11 Panwan Differ floar A02-488-51-11-15-552-012 Almond - A02 Kibal Stelestratil Hard-shell Almonds	Parwan - AF Kabul, Mandawi AF	Long 68 1062800 X Lat: 25,0505900 X Long 69,10921400 X	x x	x x x x	6.52 X X 7.34 X	5.68				T7X60	< 100			<100		
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Appendix IX –

Metabolites detected with fungal genus that commonly produces the

Commodity	Fusarium metabolites	Alternaria metabolites	Aspergillus metabolites	Penicillium metabolites
	Beauvericin	Tenuazonic acid	3-Nitropropionic acid	Mycophenolic acid
	Enniatin A	Alternariol	Kojic acid	Agroclavine
	Enniatin A1	Alternariolmethylether	Sterigmatocystin	Chanoclavin
Wheat	Enniatin B	Tentoxin	Methoxysterigmatocystin	Elymoclavine
wheat	Enniatin B1	Altersetin	Averantin	Citrinin
	Epiequisetin	Altersolanol	Averufin	Secalonic acid D
	Equisetin	Altertoxin-I	Norsolorinic acid	Questiomycin A
	Chrysogin	Macrosporin	Cycloaspeptide A	Quinolactacin A
	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
Nuts	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
		Tenuazonic acid	Malformin A	Mycophenolic acid
		Alternariol	Malformin A2	Mycophenolic acid IV
		Alternariolmethylether	Malformin C	Quinolactacin A
		Altersetin	Pyranonigrin	Andrastin A
Grapes		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 Utraviolet 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





In the name of Allah

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

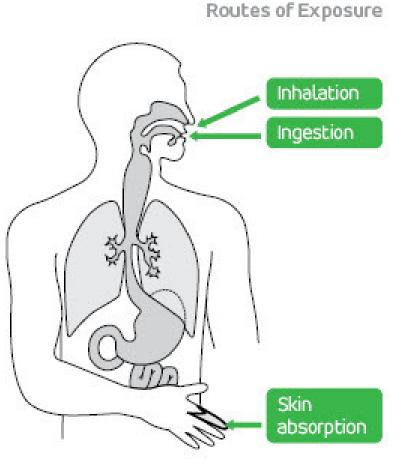


General Information

- 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



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

indirect exposure

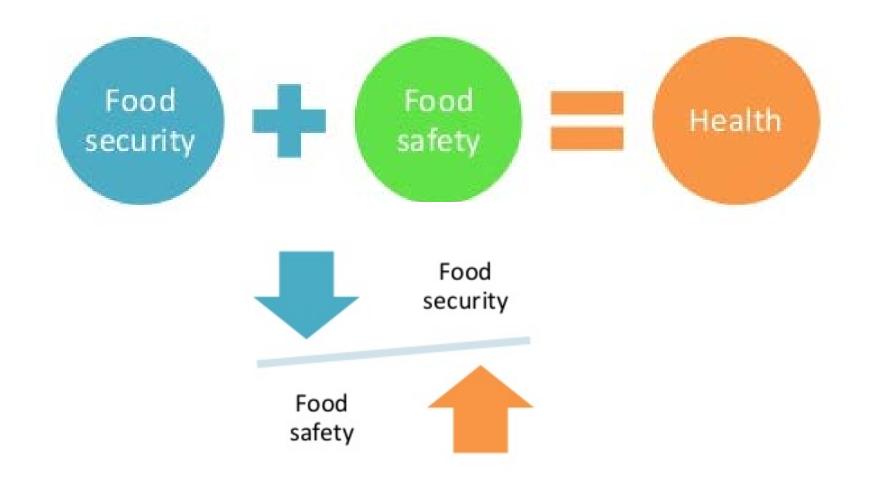
exposure

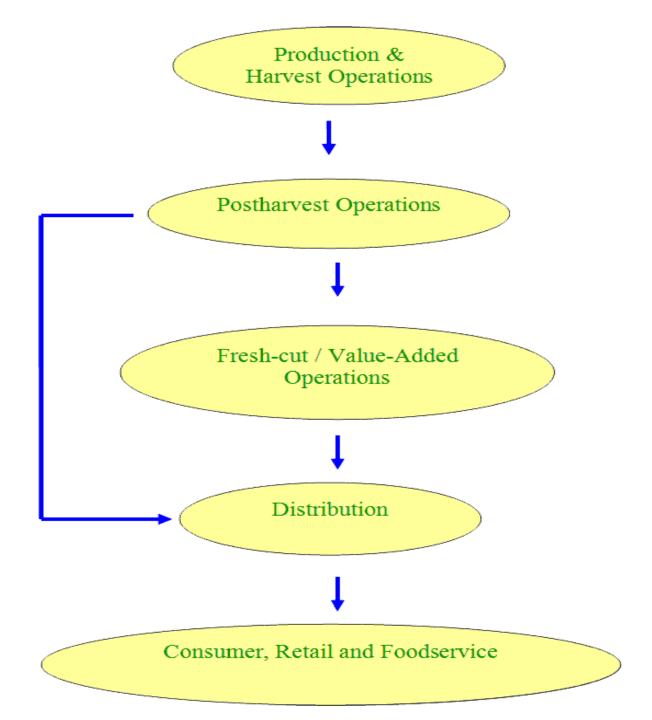
- Food Ingredients
- Residues in animal products milk, eggs, edible organ tissues





Mycotoxins: a multi-disciplinary issue









Presented by: Asadullah Ansari

MSc Plant Pathology

مقدمه Preface

Afghanistan depends on agricultural products.

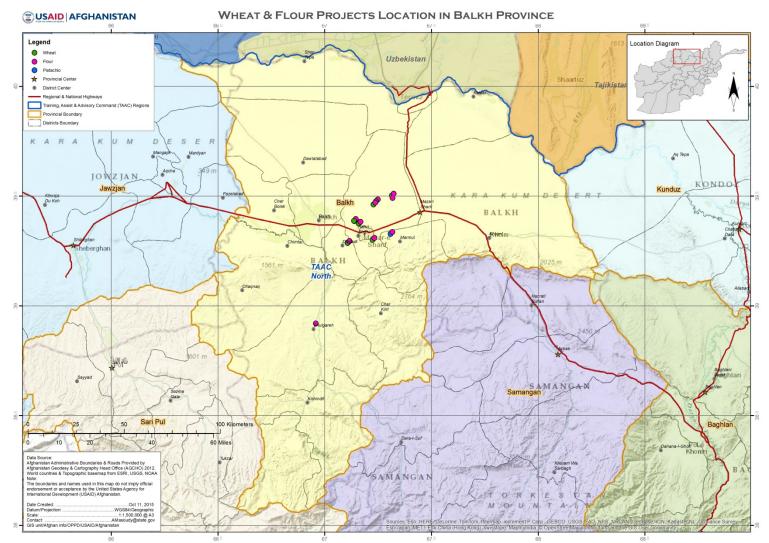
- Main source of national income.
- 85% people directly and indirectly involve in agriculture.
- Plant production is the back bone of Afghan export

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.





Objective

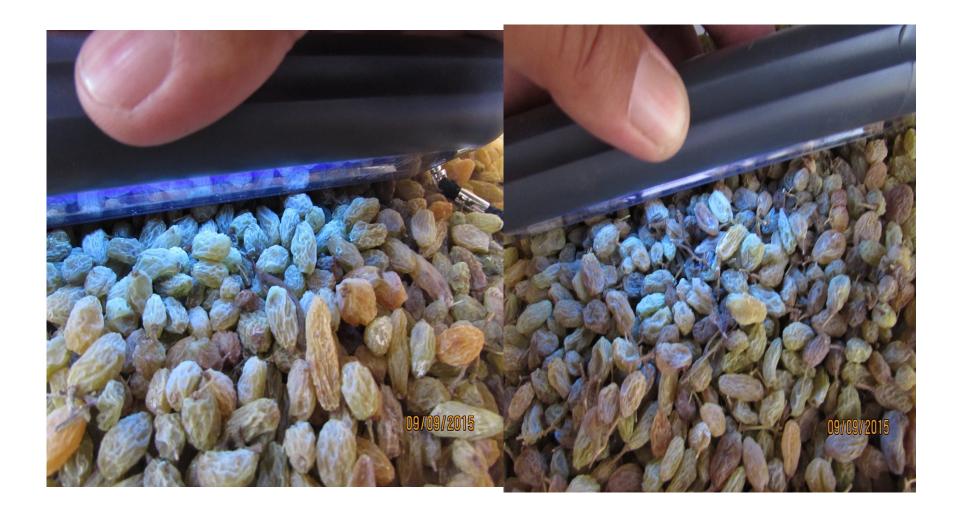
Sample collection for research on mycotoxins.

- Storage observation .
- Local standards.
- Storage duration.
- Storage sanitation.

Pre-Sampling preparation

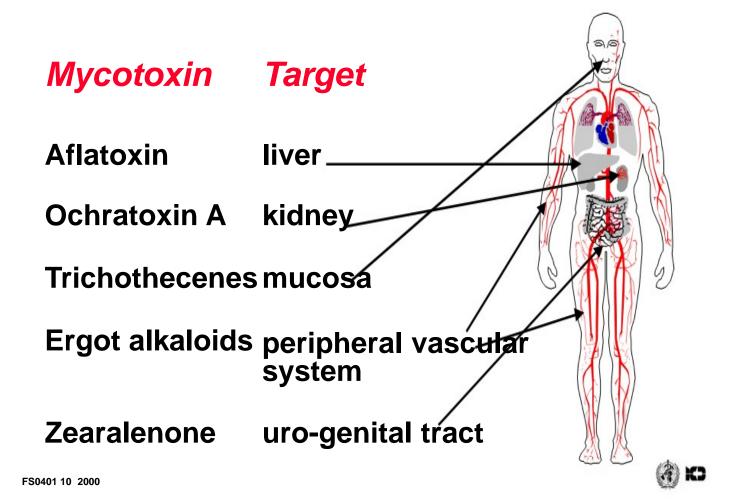
- 1. Selection area.
- 2. Equipments collection bags, gloves,
- Mask, transferring bag, UV-light, sample ID form, pen, camera, wet tissue paper, beaker, sample stick, GPS.







Target organs of some myc









مايكوتاكسين (Mycotoxin)

مايكوتاكسين (Mycotoxin):

Aflatoxin (A.parasiticus Aspergillus flavus

Ochratoxin (<u>Aspergillus ochraceus</u>, <u>Aspergillus</u>) <u>carbonarius).</u>

(T2) Fusarium sp

DON(*F. graminearum* and *F. culmorum*





Mycotoxin, Analyses procedure.

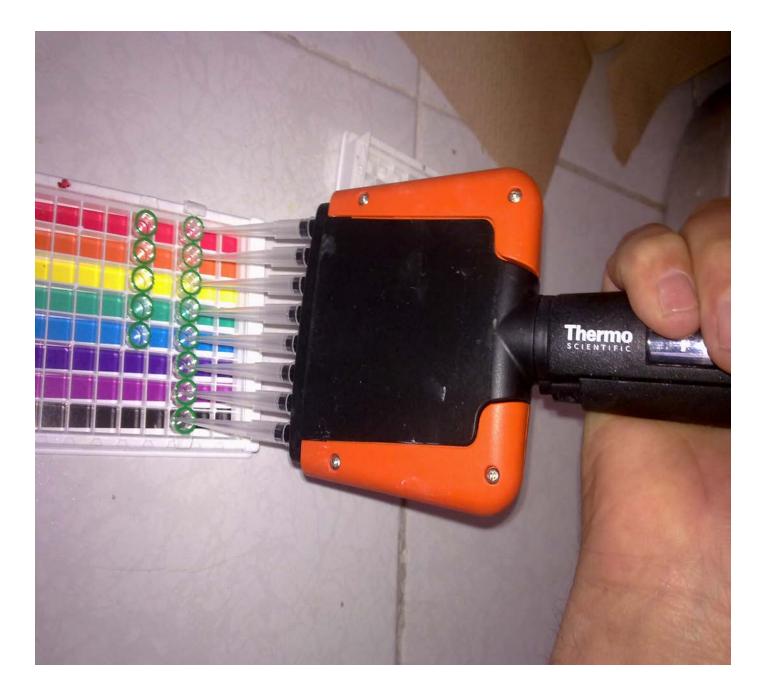
- 1-300 g of sample
- 2-450 Distilled water for raisins and nuts
- 3- MIX, 5 minutes, 20g Extract + 100μl Methanol 70%. 3minutes, MIX - Filtration



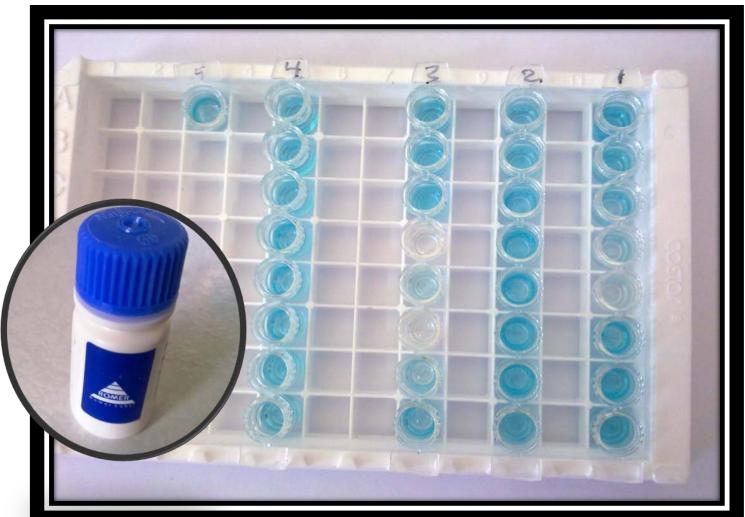


4- Elute pH is measured, ready for analysis, 4000μl
Elute, Filtration, 200μl conjugate, 100μl standards,
and 100μl Elute. MIX, Transfer 100μl, Antibody well.
15M, 5times wash, 100μl substrate 5 minutes
incubation, 100μl stop solution.





 Pipette 100 µL of the Substrate into each microwell strip using an 8-channel pipette.
 Incubate at room temperature for 5 minutes.



 Pipette 100 µL of Stop Solution into each microwell strip using an 8-channel pipettor. The color should change from blue to yellow.





فورم نتايج OD



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East the Future	Innovation Lab for the Reduction of Post-Harvest Loss
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End of Run

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, μg/kg **Max. permissible level** by FDA: 20.0 ppb, μg/kg

Raisins and

Afghanistan exporting Raisins Nut's to 50 counties

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	
Tota		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		
Tota	I	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

Afghan Raisin Fruits & Vegetables promotion administration
 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

The end

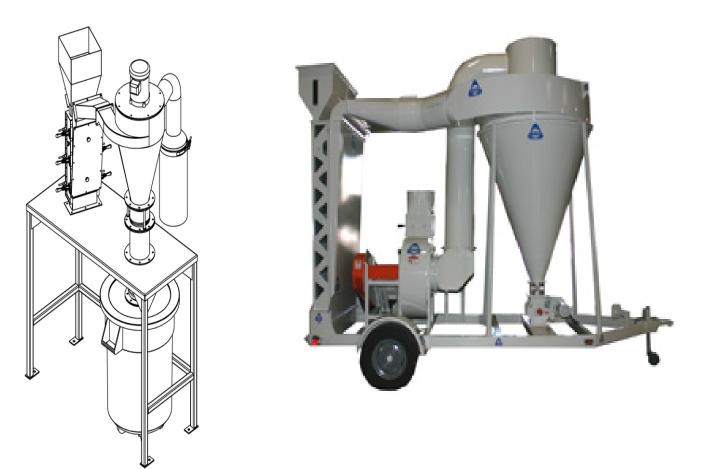
HOPE TO HAVE SAFE FOOD FOR EVERY ONE

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 Laboratories). 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 counties 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 selfregulation 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 longterm 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 HAACP (Hazard Analysis Critical Control Point) was introduced as a framework in which control of mycotoxins in food-stuffs 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
- California guidelines from Adapt GAP the 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 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.

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

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 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 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

	14 March 2016 Policy Group Schedule	
Time	Topics	Presenter/ Facilitator
14:00 – 15:00	Site Visit A: Food Safety & Standards Authority <u>http://www.fssai.gov.in/</u> FDA Bhawan near Bal Bhavan, Kotla Road	McDonald Homer
15:30 - 16:30	Site Visit B: Presentation by CHAMP team at Imperial Hotel	H. Hamid Safi,
17:00 – 18:00	<u>http://rootsofpeace.org/usaid-</u> champ_india_office_ribbon_cutting_175x_150_q50	USAID/OAG
18:00 – 19:00	 Group Session 4: Trade and Health Issues Mycotoxins & Health – Ahmed Kablan Mycotoxin Regulations – Antonio Logrieco 	John Floros
19:30	Dinner at Imperial Hotel	

Time	Topics	Presenter/ Facilitator
13:30-14:45	 Technical Issue Presentations 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 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 Mycotoxins & Health – Ahmed Kablan Mycotoxin Regulations – Antonio Logrieco 	John Floros
19:30	Dinner at Imperial Hotel	

14 March 2016 Technical Group Schedule

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 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 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 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 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? Possible responses to the problem – John Leslie & John Floros 	Ahmed Kablan
09:30 – 11:00	 Group Session 11: Nominal Group Session – The Future 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	





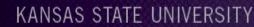
Presented at the USAID Conference on Food Quality and Safety

Delhi, India, March 13-15, 2016

John D. Floros, PhD Professor of Food Science & Engineering Dean of Agriculture & Director of KSRE Kansas State University Manhattan, KS 66506, USA



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ACCOMPLISHMENTS AT A GLANCE

Welcome to the Conference on Food Quality and Safety



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WE ARE DEDICATED TO A SAFE, SUSTAINABLE, COMPETITIVE FOOD, FEED, FIBER AND FUEL SYSTEM

TO STRONG, HEALTHY COMMUNITIES, FAMILIES AND YOUTH

THROUGH INTEGRATED RESEARCH, ANALYSIS AND EDUCATION











In Kansas

AGRICULTURE LEADS

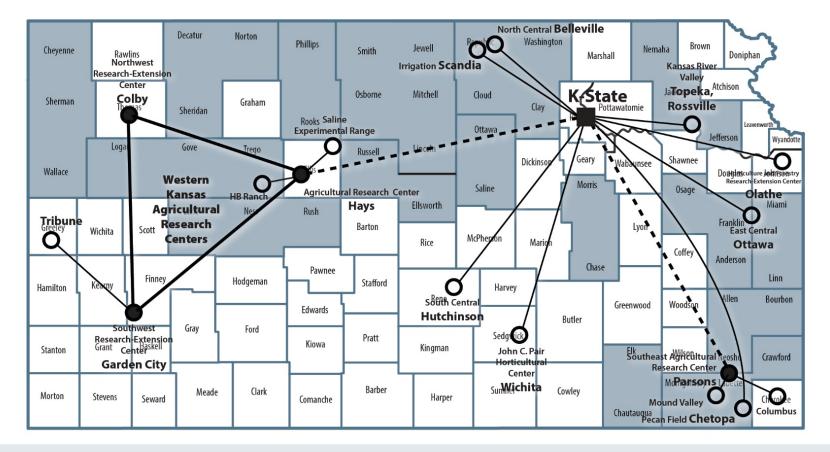








SERVING THE ENTIRE STATE OF KANSAS





College of Agriculture



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:







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



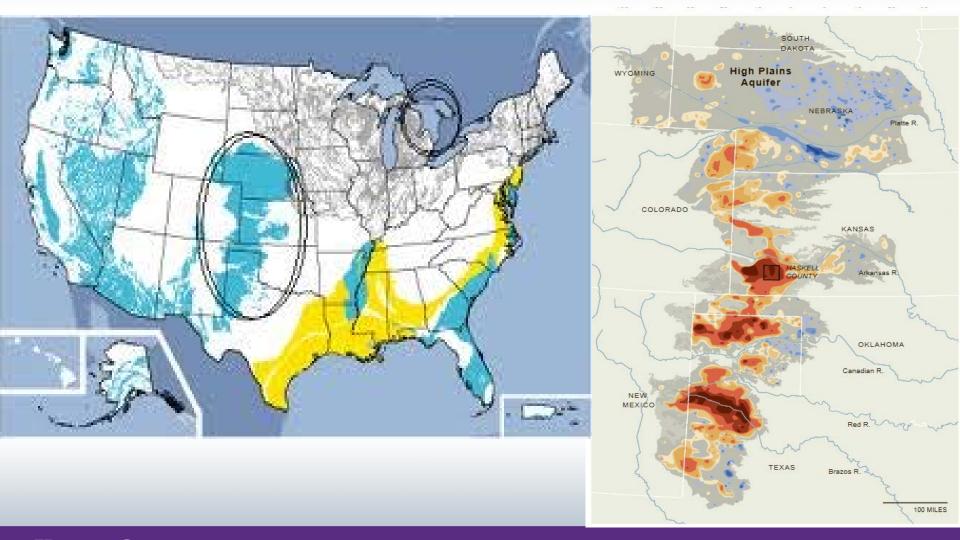


Over 2.5 Trillion M³ of water is consumed by the global agricultural sector each year





Ogallala Aquifer – An Underground Pool Drying Up



KANSAS STATE College of Agriculture IVERSIT

UN





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

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Improved harvesting systems in developing nations must be supported by efficient storage, processing and distribution systems

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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.

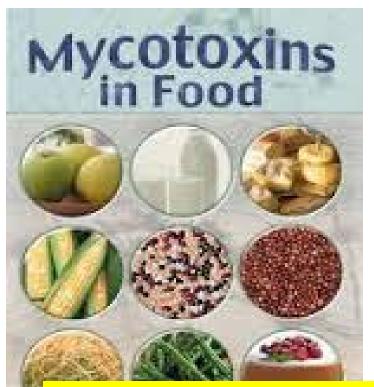
An appropriate technology approach. The technology employed does not have to be high-tech. Adoption of simple techniques (e.g. use of raised beds in agriculture, properly drying grains and legumes in order to reduce Alfatoxin) can also have a great and sustainable impact.

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



Affects a wide range of food; grains, vegetables, livestock food chains





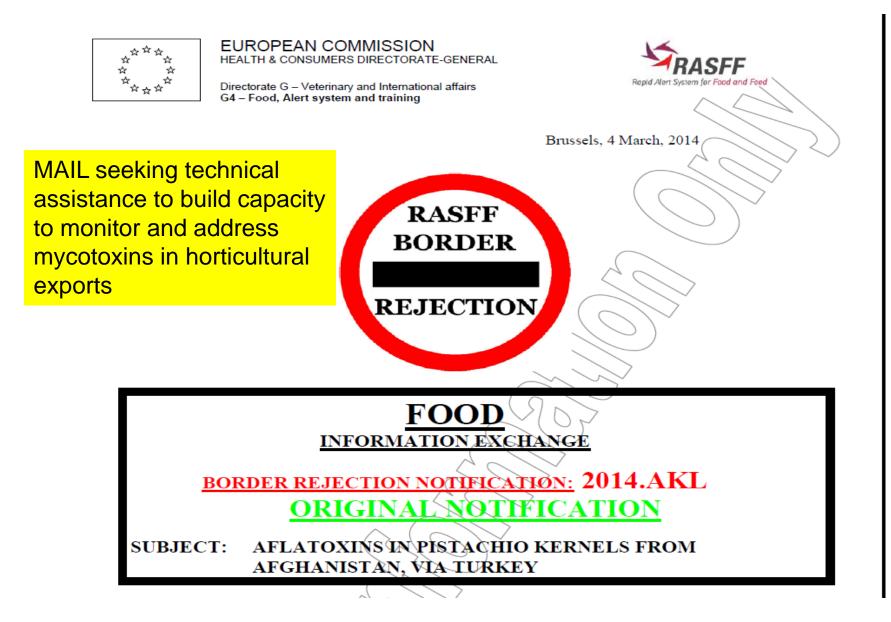
Myco=fungi; various types of fungi—different toxins, e.g. Aflatoxins, Fumonsisins

- Kidney failure
- Liver cancer
- Growth Stunting
- Immune deficiency or suppression
- Financial losses
- Nutritional Impact











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:

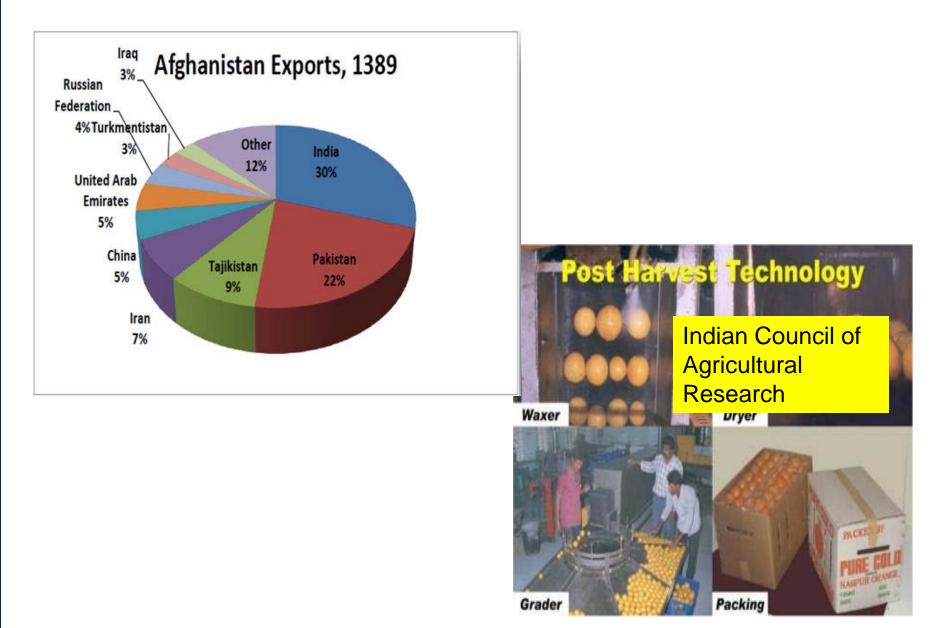
- Assessment
- Technology Transfer (field test kits, ELISA)
- Technical Capacity Enhancement (MAIL)
- Equipment/Supplies
- Information Dissemination
- International Conference
- Public-Private Partnerships
- USAID review/application of findings for current and new programs



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.









• 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

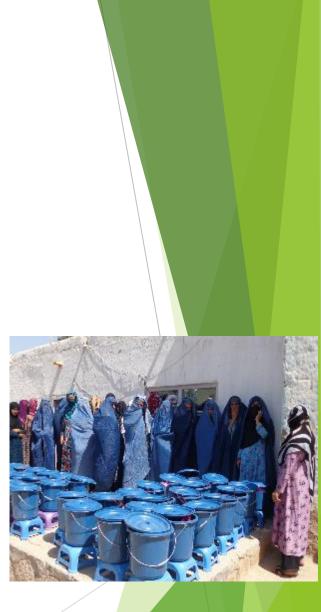
- 1. National Horticulture and Livestock Project
 - Budget : USD 100 million with additional USD 90 million to be added by end of April 2016, (USD 28 million beneficiaries contribution) total USD 2018 M
 - Closing date : December 31, 2020
 - Coverage: 26 provinces (to be expanded to 34 provinces, if 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 hectors of new commercial orchards have been establishment with intercropping)
 - Improved technologies/practices for rehabilitation of existing orchards (over 75,000 hectors)
 - 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 multistakeholder 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!

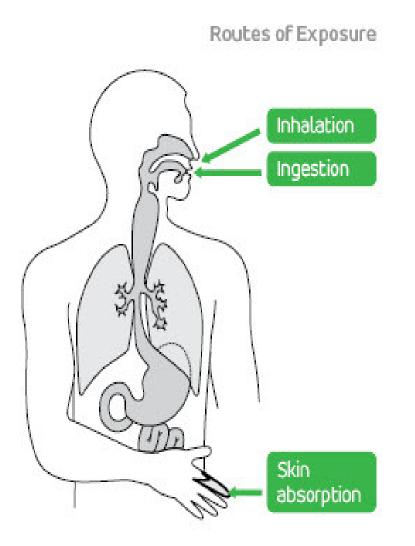




Mycotoxin impact on

> Public Health

Plants , plant products (export & import)





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)				2014 (cso)			
NO	Product	Quantity(Tons)	Cost (US\$)	NO	Plant Name	Quantity (Tons)	Cost (US \$)	
1	Red Raisins	1,012,871	19,244,563	1	Shelled Almonds	1,924	4,602,196	
2	Green Raisins	758	1,667,705	2	Almond Kernels	1,661	1,2617,000	
3	Black Raisins	3,716	5,203,751	3	Pistachio Kernels	16,311	22,517,000	
4	Golden Raisins (Abjosh)	4,525	11,260,397	4	Walnut Kernels	2,648	9,048,107	
Tota	l	1,021,870	37,376,416	Tota	I	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)

 \succ Mitigation :

➤Verification :⁹

Mitigation (Goal):

For reduction, what we need to do?

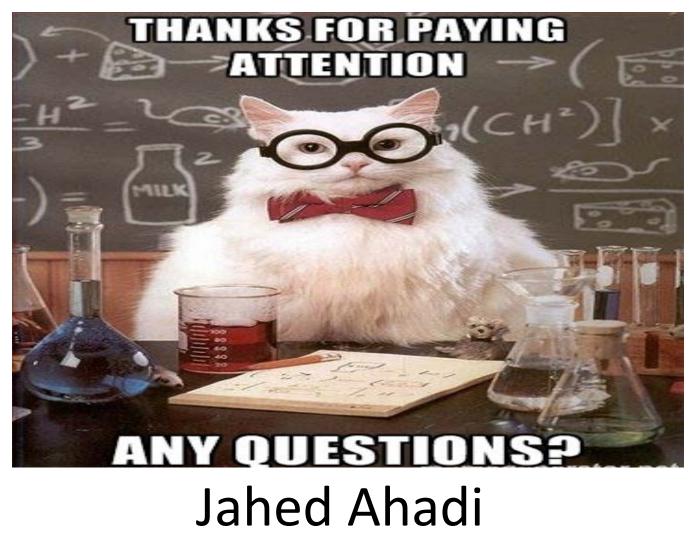
➢Install, standard raisin storage (khishmishkhana).

≻Air aspiration for nuts & grains.

► Wash product with chlorine?

Increase awareness of traders and farmers.

➢Apply for ISO. Develop and adapt support documentation



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:
 o Aflatoxins
 - o Fumonisins
 - Deoxynivalenol and other trichothecenes, e.g., T-2
 - o Zearalenone
 - o 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

What are Mycotoxins?

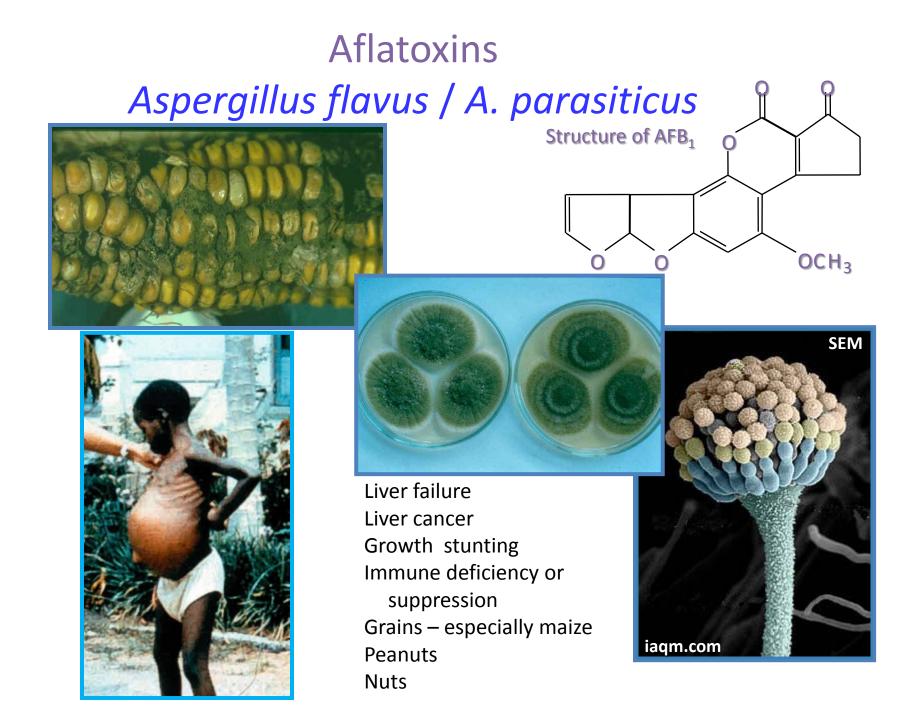
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 - o Zearalenone
 - **O Ochratoxin**
 - Ergot Alkaloids

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



Ochratoxins Aspergillus ochraceus

Kidney failure

Cacao

Grapes

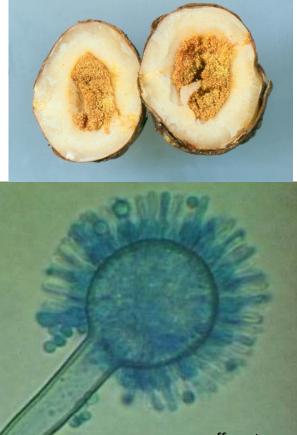
Coffee

Wheat

Nuts







coffee-ota.org

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	16 10 0 2 2 11 R1
DON	–OH	-H	–OH	–OH	=O	9 11 2 3
3-ADON	–OAc	-H	–OH	–OH	=O	
15-ADON	–OH	-H	–OAc	–OH	=O	R5 ¹¹¹ 8 7 6 5 12 4
NIV	–OH	–OH	–OH	–OH	=O	R_{5} 7 5 4
T-2	–OH	–OAc	–OAc	-H	–OIsoval	R_{4}^{15} R_{2}^{15}
HT-2	–OH	–OH	–OAc	-H	–OIsoval	
4,15-DAS	–OH	–OAc	–OAc	-H	-H	R ₃

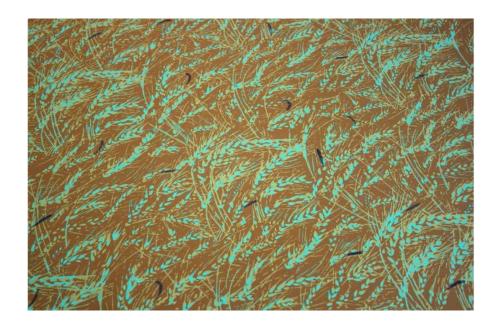


T-2 – Killed 1000s in Russia after WWII
DON – Becoming very widespread in US and Europe, especially where wheat and maize are grown
DON is changing the economic landscape of the US Great Plains *Fusarium* is the main producer on grains, but other fungi and some plants also synthesize

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



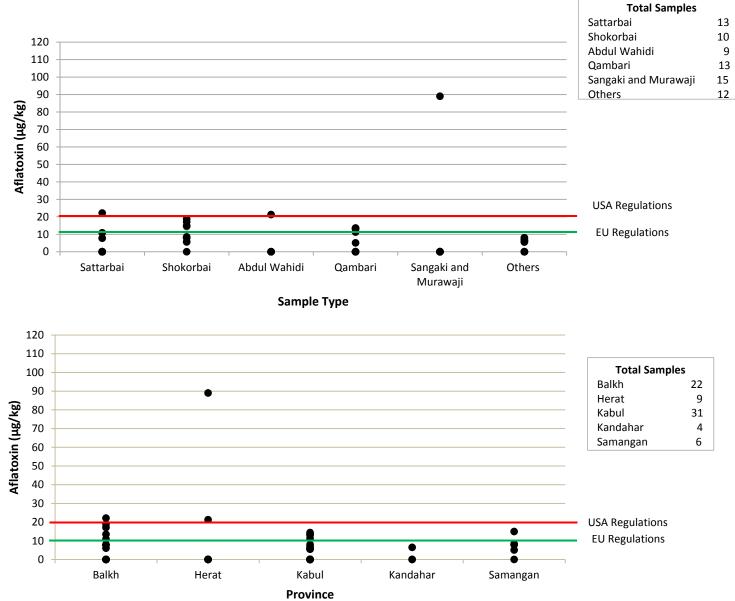
Test Kit Performance

- 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

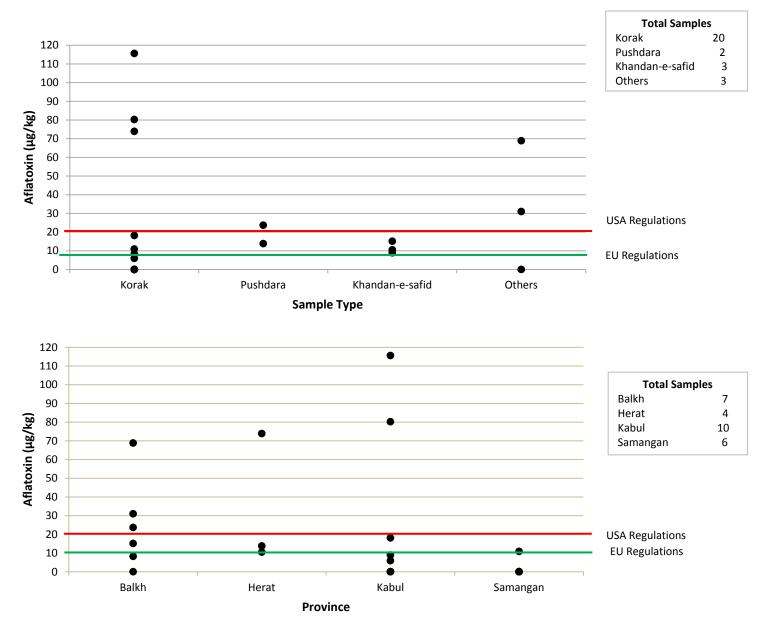
Nuts – Results

- 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

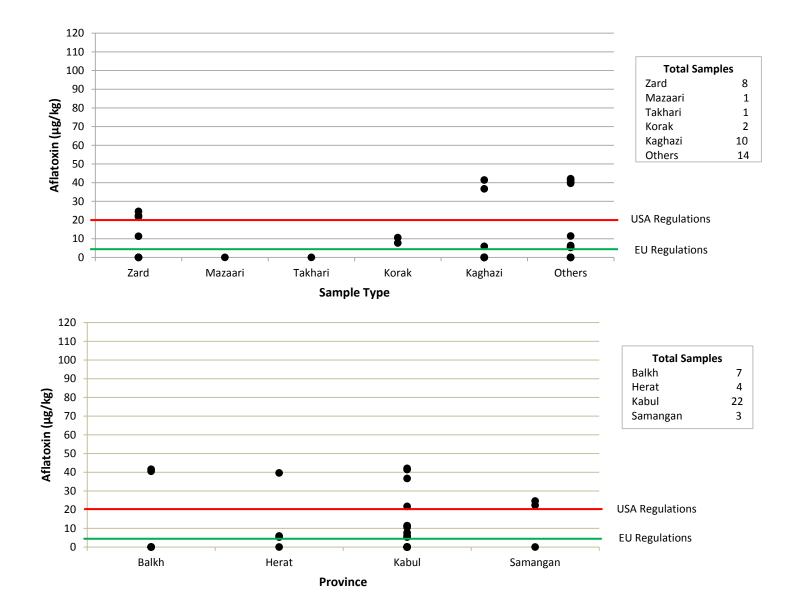
Almonds – Aflatoxins



Pistachios – Aflatoxins



Walnuts – Aflatoxins



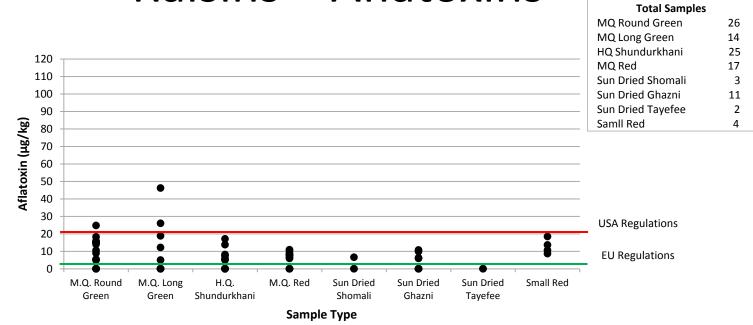
Austrian Screen – Nuts

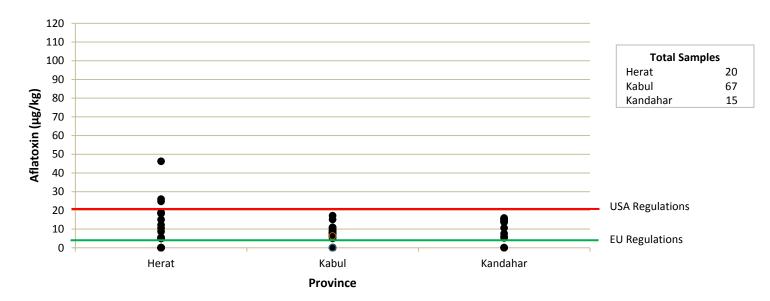
Fusarium	Alternaria	Aspergillus	Penicillium
Butenolide	Alternariol	Cyclopiazonic Acid	Andrastin A
	Alternariol methyl		
Epiequisetin	ether	Aflatoxin	Andrastin B
Equisetin	Altersetin	Asperfuran	Agroclavine
Fusaric acid	Infectopyron	Kojic acid	Chanoclavin
HT-2 toxin	Macrosporin	Malformin A	Epoxyagroclavin
T-2 toxin	Tentoxin	Malformin A2	Festuclavine
Zearalenone	Tenuazonic acid	Malformin C	Mycophenolic acid
			Mycophenolic acid
α-Zearalenol		Nigragillin	IV
β-Zearalenol		3-Nitropropionic acid	Penitrem A
		Ochratoxin	
		Paspalin	

Raisins – Results

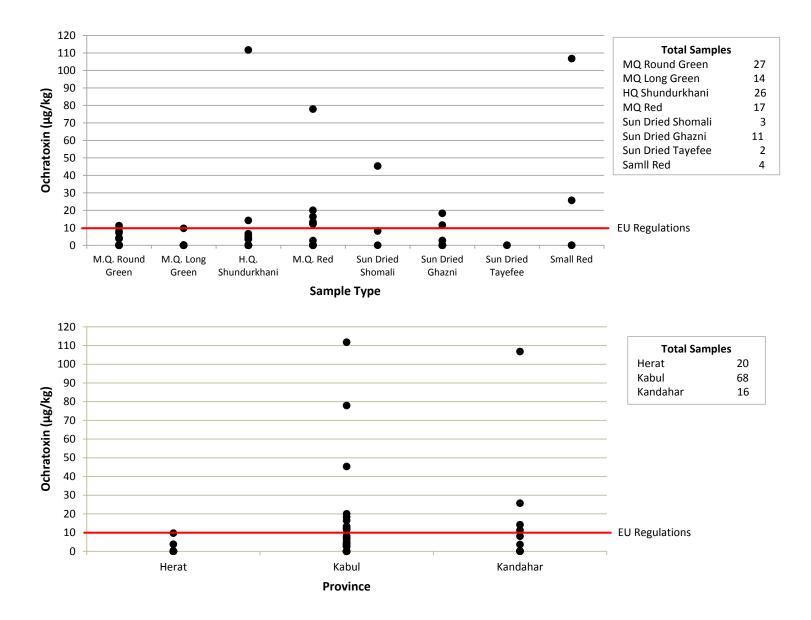
- Aflatoxins could limit exports in 43/89 samples
- Ochratoxin could limit exports in 25/80 samples
- Raisin type and drying method can be important
- Afghanistan and Austria results are discordant
- Choice of country to export to may depend on level of contamination

Raisins – Aflatoxins





Raisins – Ochratoxin



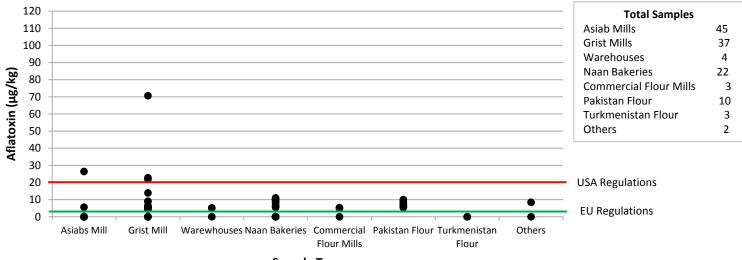
Austrian Screen – Raisins

Fusarium	Alternaria	Aspergillus	Penicillium	
Fumonisins	Alternariol	Aflatoxin	Andrastin A	
	Alternariol methyl ether	Aurasperon B	Andrastin B	
	Altersetin	Aurasperon C	Andrastin C	
	Altertoxin-I	Aurasperon G	Chanoclavin	
	Macrosporin	Fonsecin	Festuclavine	
	Tentoxin	Malformin A	Mycophenolic acid	
	Tenuazonic acid	Malformin A2	Mycophenolic acid IV	
		Malformin C	Penitrem A	
		Nigragillin	Quinolactacin A	
		Ochratoxin		
		Pyranonigrin		

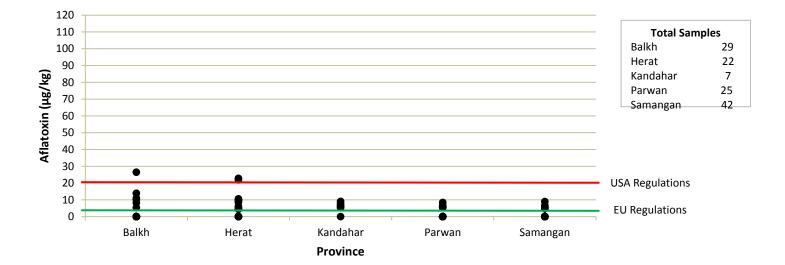
Wheat – Results

- 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

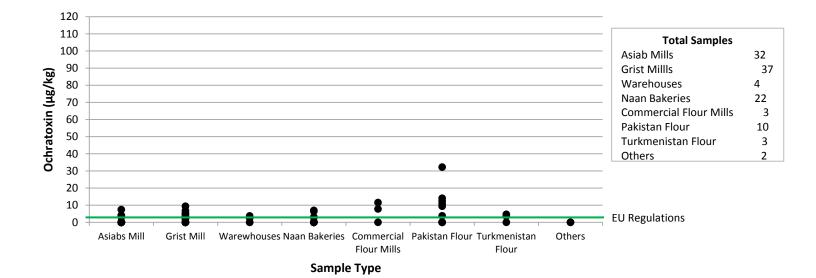
Wheat - Aflatoxin

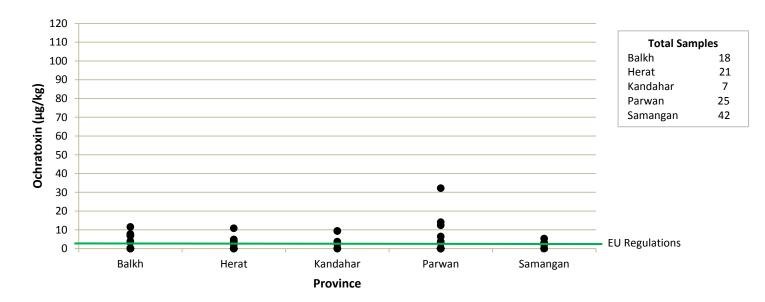


Sample Type



Wheat – Ochratoxin





Austrian Screen – Wheat

Fusarium	Alternaria	Aspergillus	Penicillium	Claviceps
Beauvericin	Alternariol	Aflatoxin	Agroclavine	Ergocristine
	Alternariol			
Enniatin A	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
		Methoxysterigm	Mycophenolic	
Epiequisetin	Macrosporin	atocystin	acid	Ergosinin
		3-Nitropropionic		
Equisetin	Tentoxin	acid	Questiomycin A	Ergotamine
	Tenuazonic		Quinolactacin	
HT-2 toxin	acid	Norsolorinic acid	А	Ergotaminine
			Secalonic acid	
T-2 toxin		Ochratoxin	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

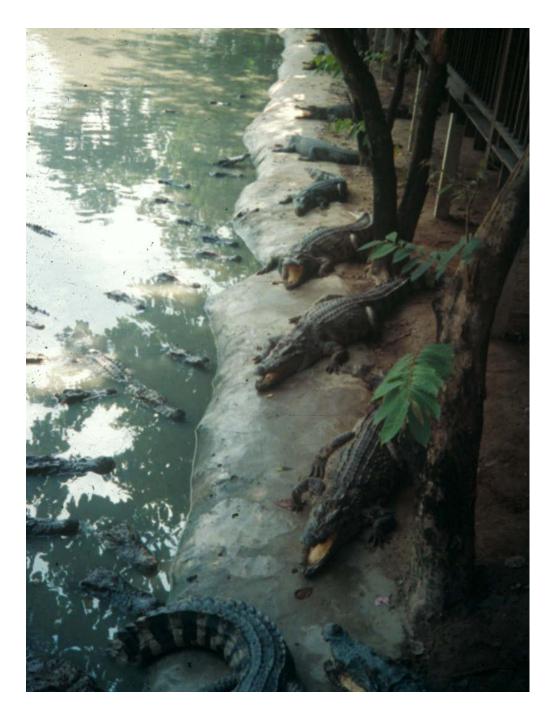


Special thanks to all of our collaborators in Afghanistan, Austria, Nebraska, Kansas (and my wife!).

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.





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

Feed the Future Innova Reduction of Post-H Individual sample in Name: Sample identification:	larvest Loss	
Individual sample in	formation	
	Phone:	
Sample identification:		
	- sampler - date - sequential number	
		in the
Sample description:	Nuts Raisins	
Date of sample collection:	ruis ruisins	and the second
MM	DD YY	
Sample origin:		1.2
(Source of sample if known; e.g.: I X province or purchased at	arvested/produced at	
Sample location:		
(of sample provider, including GPS location; e.g.: Ka	bul market - L" and L ")	1
Type of storage:		1000
Storage period: (Since purchase or harvest, if known)		
Observations: _ (-) or (+)		
		Contrast -

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, USAID 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



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 identifications in several arrest several arrest is several arrest in the several arrest is a several arrest in the several arrest is a
Page I of_ KANSAS STATE Feed the Future Innovation Lab for the Reduction of Post-Harvest Loss Sampling control: Laboratory A.B. Sample receiving Responsible Sample location Sample ID Observations for sampling (provider, GPS) date (MM/DD/YY) Field 10 110/- B Sq 8-5-2015 093 36-110153817 Taked modity - province - sampler - date - sequential number 4621, 49, 4700 8-10-2015 Ruspami Lab 10 wal - 1- 12 9-19-245-020 omodity - province - sampler - date - sequential number - lab number Responsible for Date of sample Sample Sample origin Storage period Type of storage collection (MM/DD/YY) description receiving PPUD Balkhpr 8-5-2015 inside will Flour . 2 days 166 Balkh Promis VINCE Sample receiving Responsible Sample location Observations Sample ID (provider, GPS) date (MM/DD/YY) for sampling lot - 341 3211 Field ID: 461- 6-52-8-4-2015-064 bari No func 6600 8.8-2015 comodity - province - sampler - date - sequential number Zallia PESCEVER Lab 10 ald- 6-32- 8, 4-2015-064-022 Responsible for Sample Storage period Sample origin receiving description collection (MM/DD/YY) 2 days ago mgi de ghof

1692,10

8-4-2015

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.

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Printed Steel	239-007 A04-KBL-S1-10-4-15 339-007 A07-KBL-S1-10-3-15 338-006 A07-KBL-S1-10-3-15 338-006 A04-KBL-S1-10-3-15 330-008 A04-KBL-S1-10-3-15 370-008	A1 B1 A2 B2 A3 B3		Rustani		7,82 7,55 6,87 6,92		1,107	3	
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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

		reeu
	AD S	ppb Intrp
	Struff: 1 Carrier Position: Running New Curve A 51 1.429 B 52 1.284 C 53 0.922 D 54 0.466 E 55 0.116 E 55 0.116	1 0.0 4.0 10.0 20.0 40.0 0.0
1	6 2 1.520	0.0 1.2
		3377
	r=-0.9682 y=1. m=-0.0334 strip: 2 Carrier Position: A 4 -1.190 B 5 -0.002 C 6 -0.002 D 7 -0.002 E 8 -0.002 F 9 -0.001 G 10 -0.002 H 11 -0.002	
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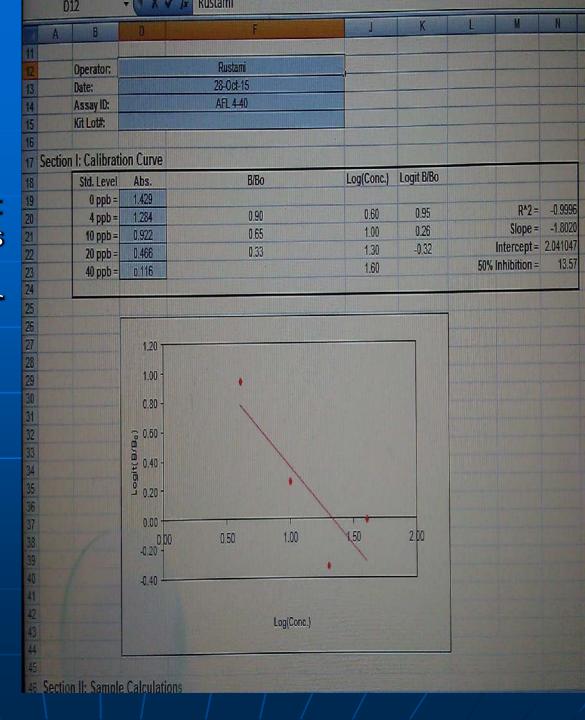


Optical Density (OD)

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 mycotoxinspecific 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,

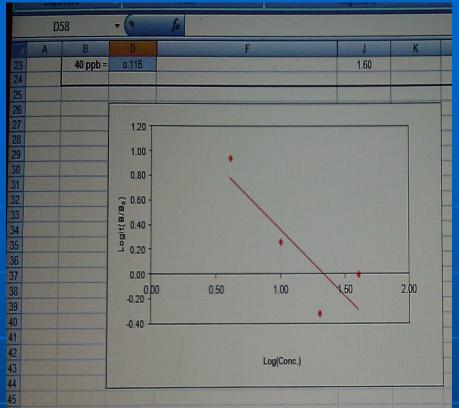


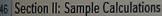
Section II

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

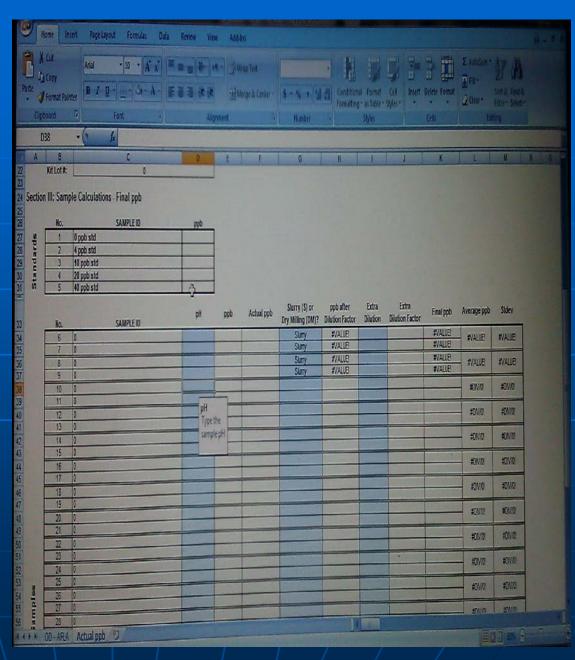




47					
47 48	No.	ABS	SAMPLE	logit B/Bo	ppb
49	1	1.429	0 ppb std	The State of the second second	
50	2	1.284	4 ppb std	0.95	4.05
51	3	0.922	10 ppb std	0.26	9.74
52	4	0.466	20 ppb std	-0.32	20.30
53	5	0.116	40 ppb std		
54 55 56	6	1.562			
55	7	1.52			
56	8	1.297		0.99	3.82
57	9	1.19		0.70	5.57
58	10				
AAPH	OD - AFLA	Actual o	nh / *]		

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

















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

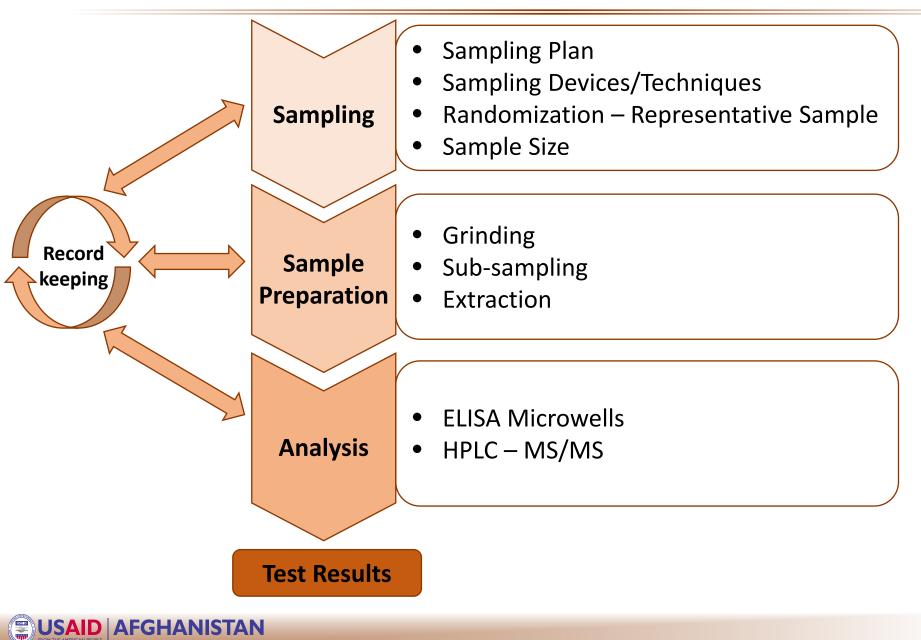
False negatives vs False positives

A crucial aspect of mycotoxin analysis is its <u>replicability</u> High replicability = low variability



\bigcirc

Mycotoxin Analysis – Main Steps



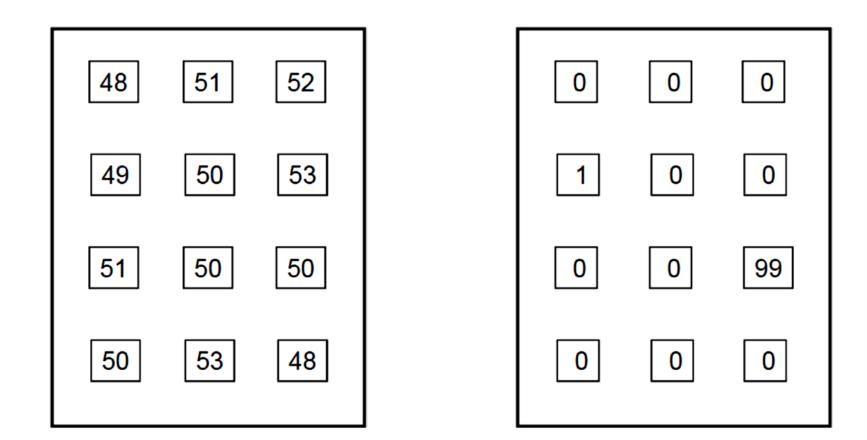


- Major source of error and variation
- Mycotoxins are not evenly distributed in a lot
- Not every kernel or nut is contaminated
- A few kernels can contaminate large lots
- "Cherry-picking"









Typical Protein Distribution

Typical Mycotoxin Distribution





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

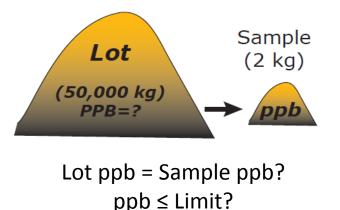






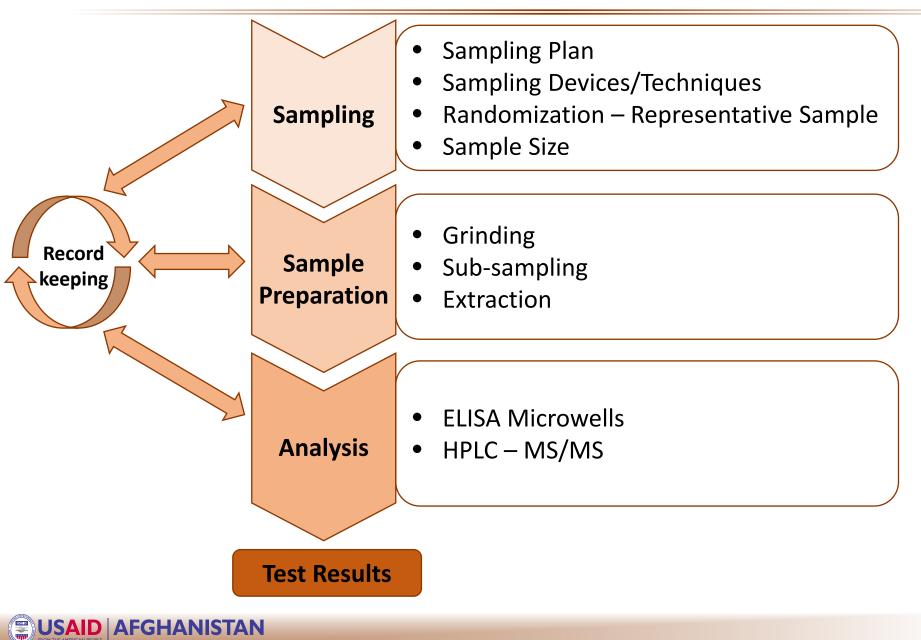
Table 1. Effect of "cherry-picking" on mycotoxin test results

Nucatavia	Commodity	Laboratory						
Mycotoxin	Commodity	Afghanistan	Austria	USA (UNL)				
	Walnut-551	25	< LOD	< LOD				
	Walnut-554	22	< LOD	< LOD				
Aflatoxin	Pistachio-612	< LOD	142	46				
(µg/kg)	Pistachio-624	< LOD	14	100				
	Almond-504	14	< LOD	< LOD				
	Raisin-269	18	< LOD					



\bigcirc

Mycotoxin Analysis – Main Steps

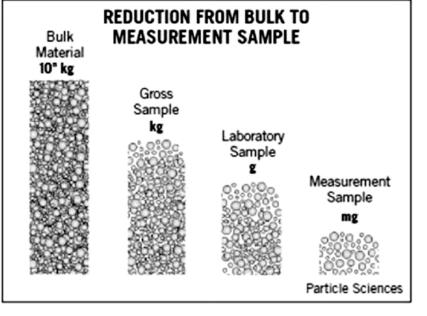




Sample Preparation

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



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





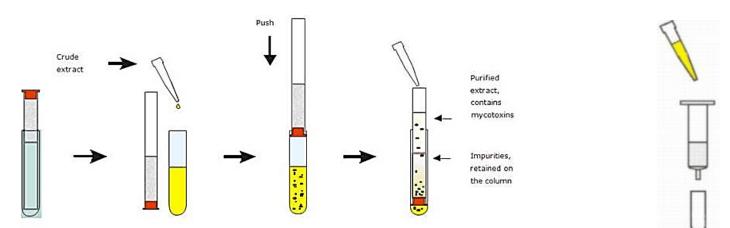
Sample Preparation

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
- \checkmark Concentrating the mycotoxins for quantification



MycoSep[®] Working Principle – Aflatoxin Analysis by Romer Labs





Sample Preparation

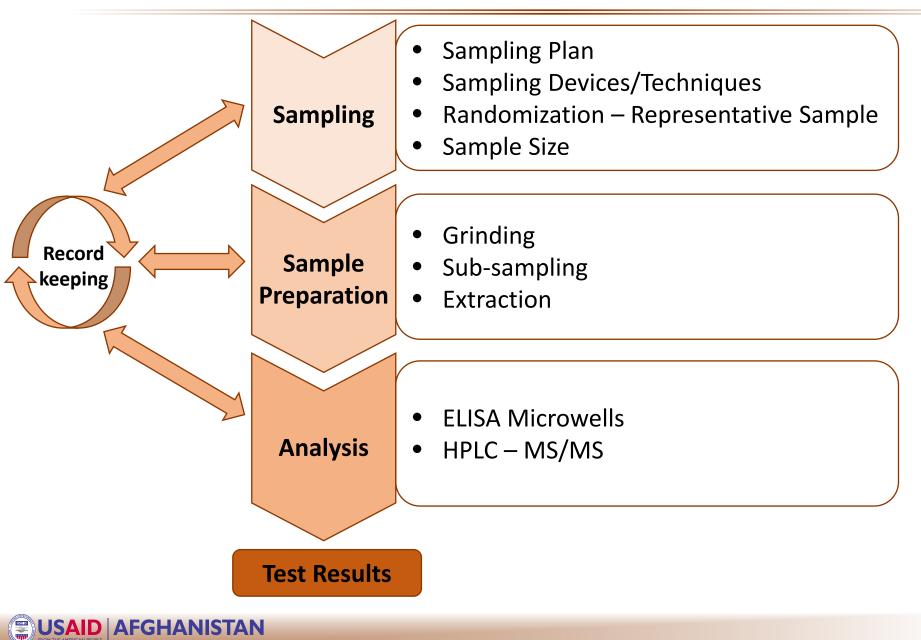
Table 2. Effect of "splitting before grinding" on mycotoxin test results

Mycotoxin	Commodity	Batch 1	Batch 2				
(µg/kg)	Commodity	Afghanistan	Austria	USA (UNL)	USA (KSU)		
	Wheat-68	5	< LOD	< LOD	< LOD		
Aflatoxin	Wheat-110	10	< LOD	< LOD	< LOD		
Anatoxin	Walnut-551	25	< LOD	< LOD			
	Pistachio-624	< LOD	15	95			
Doownivalanal	Wheat-3	3500	< LOD	< LOD	< LOD		
Deoxynivalenol	Wheat-14	1290	< LOD	< LOD	< LOD		
Ochratoxin	Raisin-296	13	< LOD				
	Raisin-302	< LOD	10				



\bigcirc

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
- $\checkmark\,$ 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

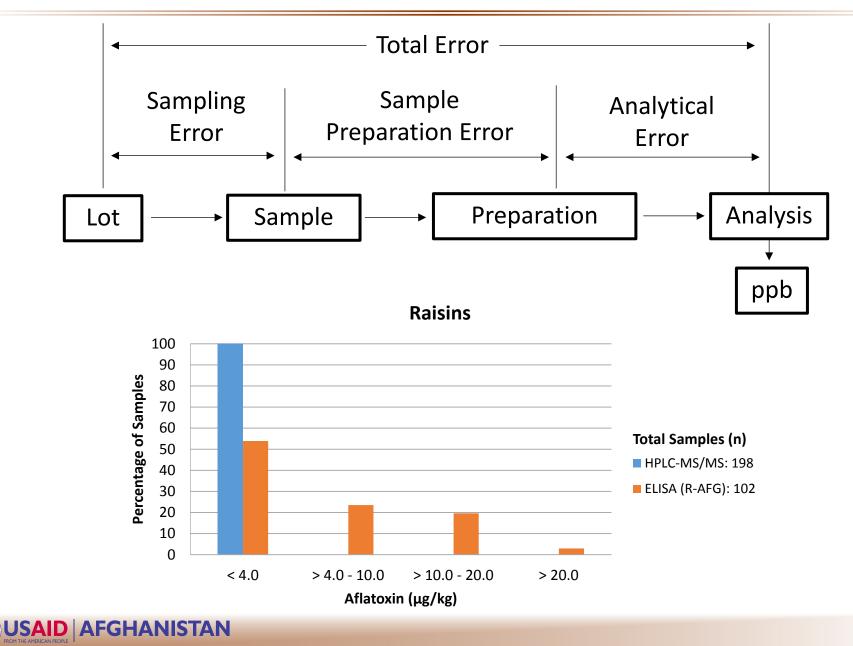
Mycotoxin	.	Analytical Method					
(μg/kg)	Commodity	ELISA-Romer	ELISA-Neogen	HPLC-MS/MS			
	Pistachio-600	< LOD	< LOD	< LOD			
	Pistachio-606	9	6	5			
	Pistachio-607	< LOD	< LOD	1			
	Pistachio-610	< LOD	20	< LOD			
	Pistachio-611	< LOD	< LOD	< LOD			
	Pistachio-612	46	29	142			
Aflatoxin	Pistachio-614	< 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			





USAID

Total Error



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

AFGHANIS

• 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

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Record Keeping

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Stop: 1 Grier Position: 1 A 1 1.678 0.0	Feed the Futu	re In		n Lab fo Control			Post-Ha	arvest Lo	55			Feed the Future Innovation Lab for the Reduction of Post-Harvest Loss A1 × B1 Individual sample information Name: Compare: 075011 Sample identification: Areas Sample identification:
B 2 1.237 2.9 C 3 0.658 20.0 D 4 0.410 27.3 E 5 0.174 34.2 F 6 1.442 0.0		ple 10	Rep A or B	Sample type	Operator	Mycotoxin	Before	oH After	OD	Additional dilution	Final OD	Sample description:
G 7 1.377 0.0 H 8 1.191 4.3				Almond	-	Flutoxi	2					Wheat Nuts Raisi
itrip: 2		SY 04 Store	603A	N	643	N	0.00					Date of sample collection:
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trip: 3	-	-										(of sample provider, including GPS location; e.g.: Kabul market - L" and L ")
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nd of Test												Observed "
							in contraction				_	Staved in Clean bags. Socies Almond.





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?





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 <u>akablan@usaid.gov</u>



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</p>
- 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 postharvest 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 Fumonisins

3 Deoxynivalenol

May share a downstream pathway for impaired growth by targeting the intestinal tract and inducing environmental enteropathy (EE)?!?!



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



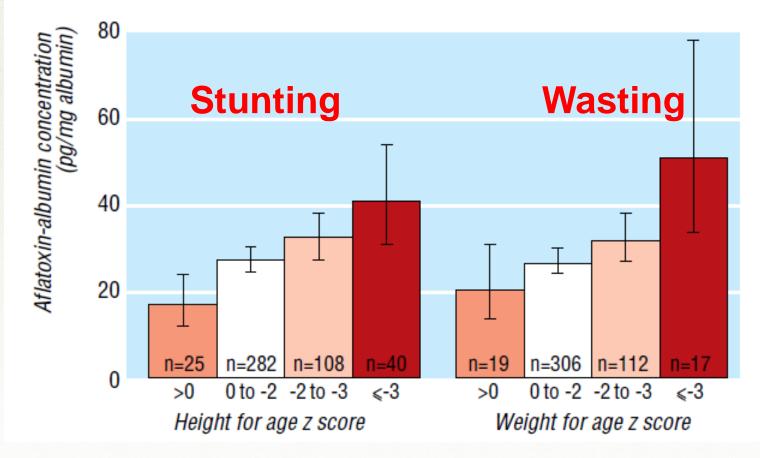
E.g. Studies linking aflatoxin to growth impairment in children-Just an example of the evidence!!

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</i> <i>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	Benin (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					

Khlangwiset P, Shephard GS, Wu F (2011). Aflatoxins and growth impairment: A review. *Critical Reviews in Toxicology* 41:740-755.



• 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

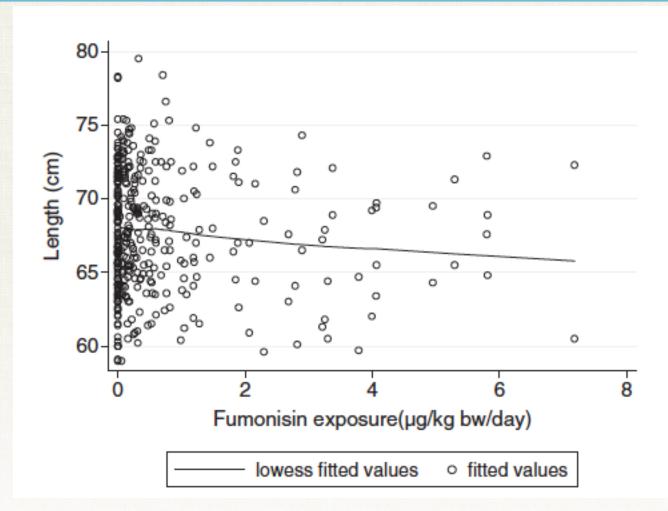
Positive correlation had been found between high maize consumption and HIV transmission ----- FUM exposure?? (FUM effect on cell membrane integrity??)



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

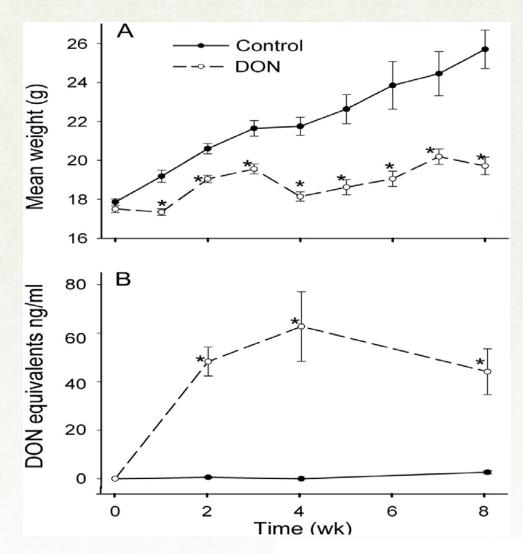


Knowledge we have from animal studies---In 1995 Rotter et al found that pigs fed grain contaminated with DON had a 20% lower feed intake and a 13% lower weight gain than the control group

Amuzie and Pestka (2010) found that DON intake in mice induced a decrease in circulating levels of IFG-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

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TOXICOLOGICAL SCIENCES



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)

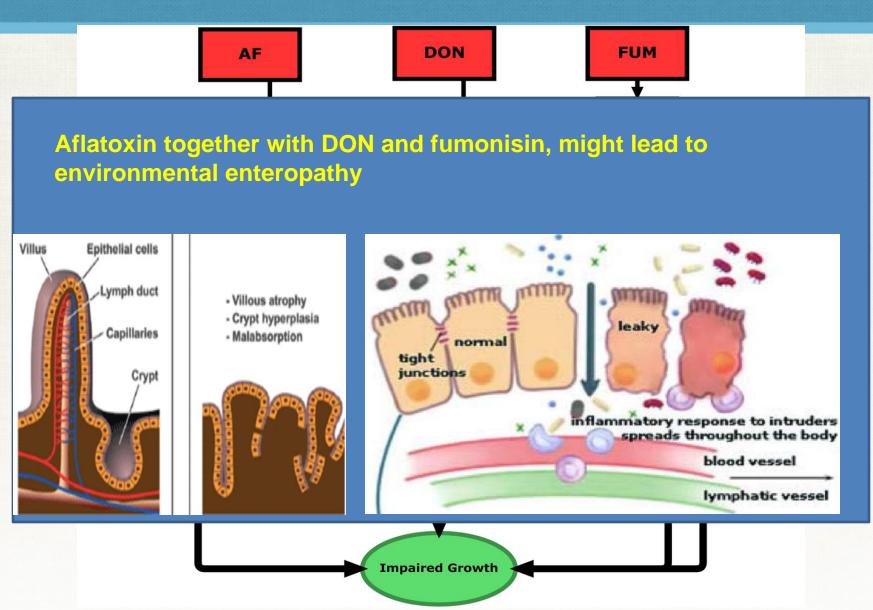
3) Downregulation of genes associated with energy production and fatty acid metabolism (Yarru et al., 2009)

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).



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Laura E. Smith et al. Adv Nutr 2012;3:526-531



Interventions to reduce aflatoxin risk

Preharvest

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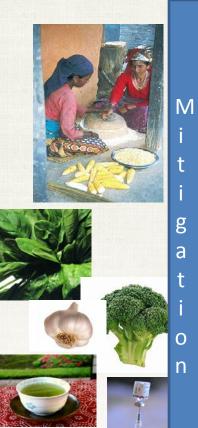
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- Good agricultural practices
- Genetically enhancing plants' resistance
- Biocontrol
- Biotechnology/breeding

Postharvest

- Improved sorting, drying, food storage
- Crops not prone to aflatoxin (e.g. Soybean)



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
- <u>Stunting is strongly related to foodborne</u> toxins (such as <u>Aflatoxin</u>), etc.



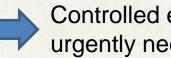
Conclusions

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

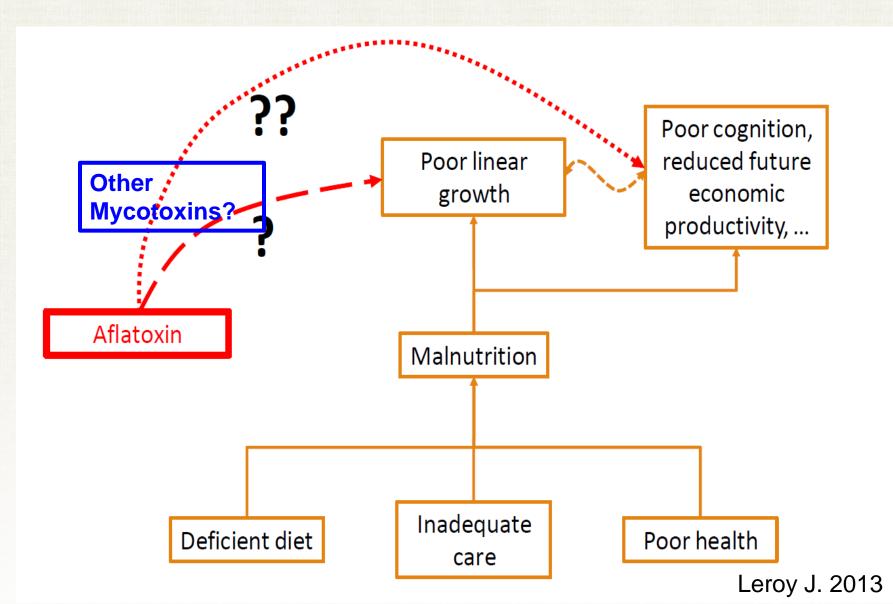
Should we wait to take an action for more evidence ?



Absolutely not; we have enough evidence from animal and human studies and we need to take actions urgently.



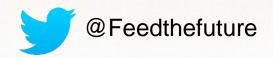








www.feedthefuture.gov



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"



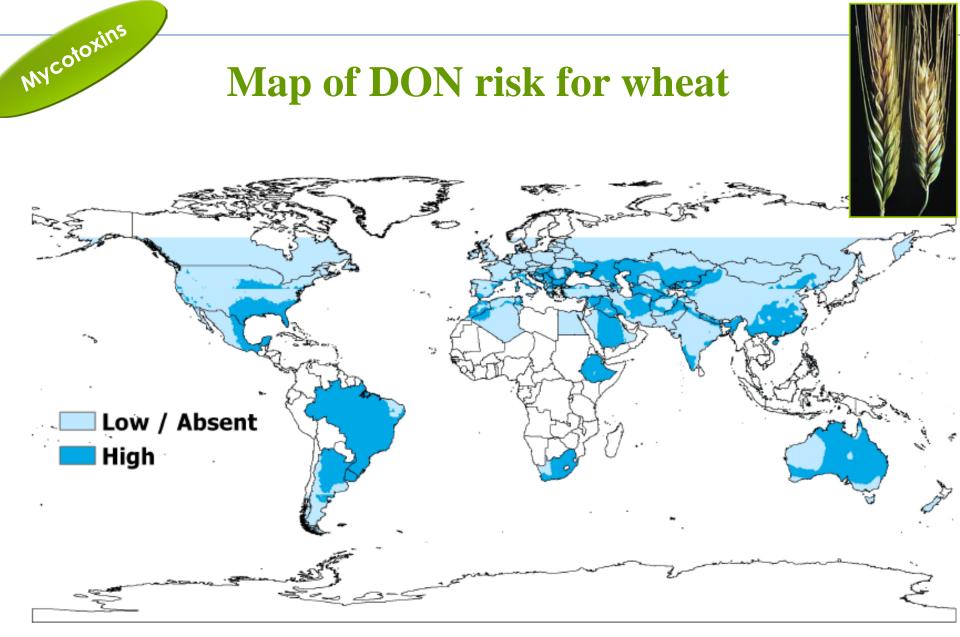
March 14-16, 2016 – New Delhi (India)

MYCOTOXIN REGULATIONS



Antonio F. Logrieco

Institute of Sciences of Food Production (ISPA) National Research Council (CNR)

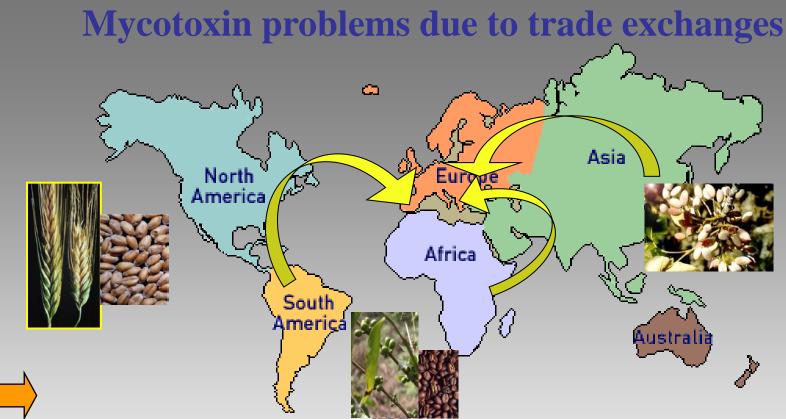


Battilani and Logrieco, 2012





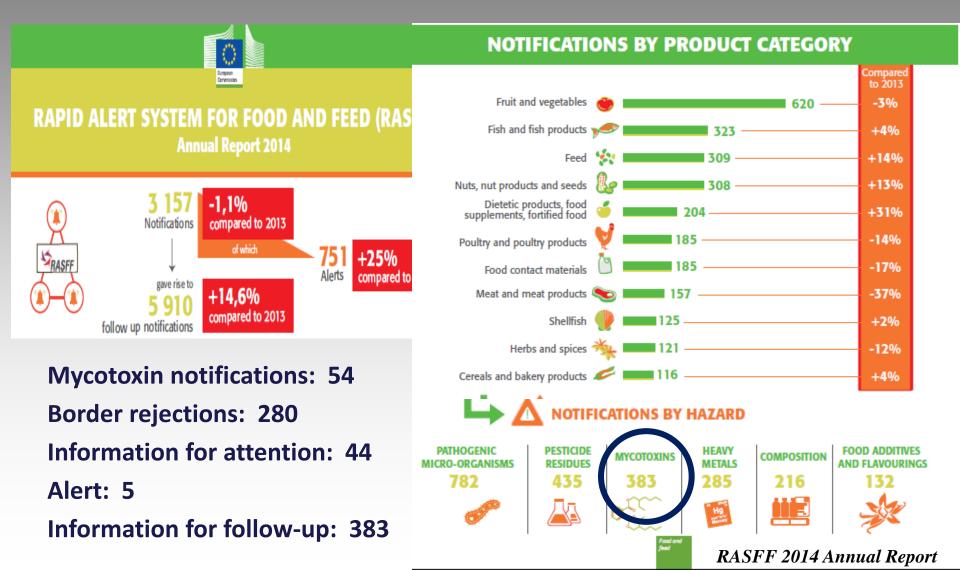




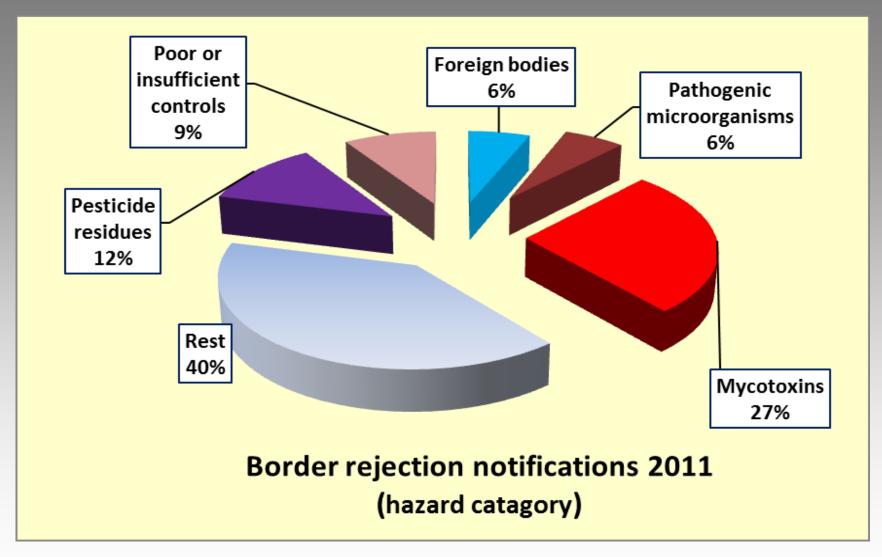
Imported products with high risk of mycotoxin contamination: •maize, (fumonisins and aflatoxins) from all continents •cereals (deoxynivalenol, ochratoxin A) mostly from north and south America •coffee, (ochratoxin A) mostly South America & Africa •pistachio nuts, (aflatoxins) mostly from North Africa & Asia •Peanuts & other nuts, (aflatoxins) mostly North, South America & Africa •Spices (aflatoxins) mostly from Asia & Africa



RASFF 2014 NOTIFICATIONS



RASFF 2011: Border rejection notifications



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
Fumonisins	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



RASFF 2013 Annual Report

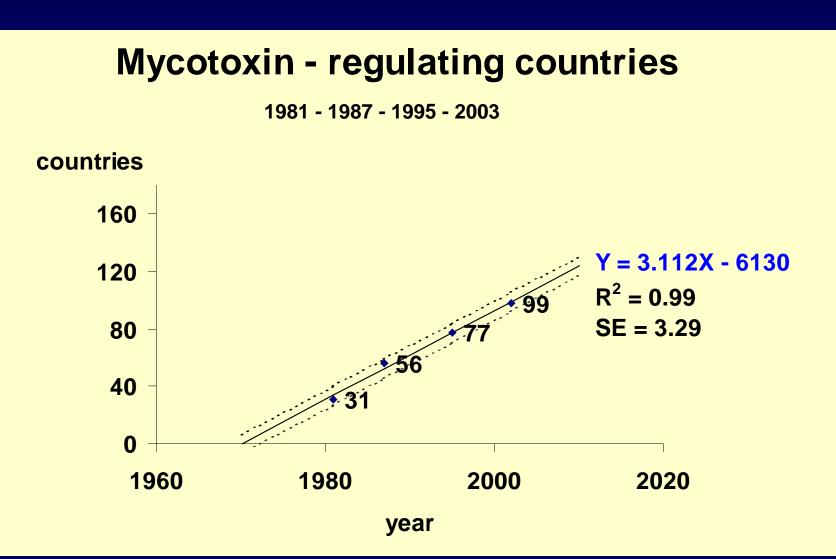
Most aflatoxin-related notifications are linked to nuts and dried fruit



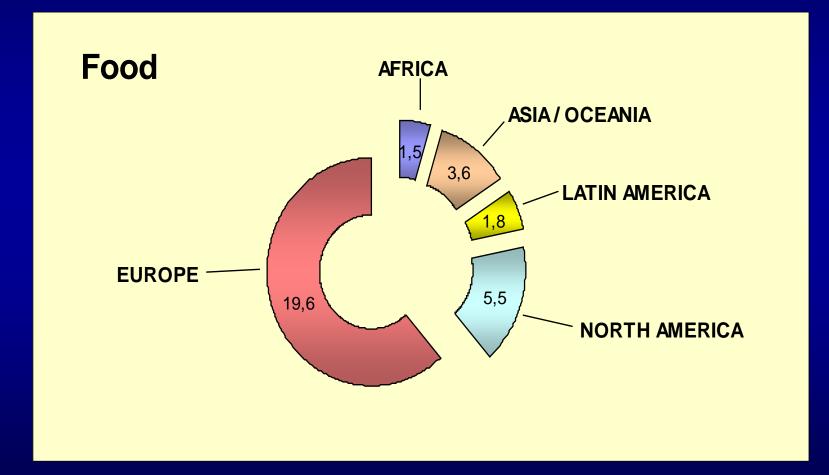
Table 5 – Aflatoxin notifications for certain products under reinforced checks regime

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



Number of mycotoxin regulations per country



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 ofcare

- EFSA panels, in coopera scientific opinions on my
- Opinions published on website and in EFSA journal



EFSA Journal 2011;9(12):2481

SCIENTIFIC OPINION

Scientific Opinion on the risks for animal and public health related to the presence of T-2 and HT-2 toxin in food and feed¹

EFSA Panel on Contaminants in the Food Chain (CONTAM)^{2, 3}

European Food Safety Authority (EFSA), Parma, Italy

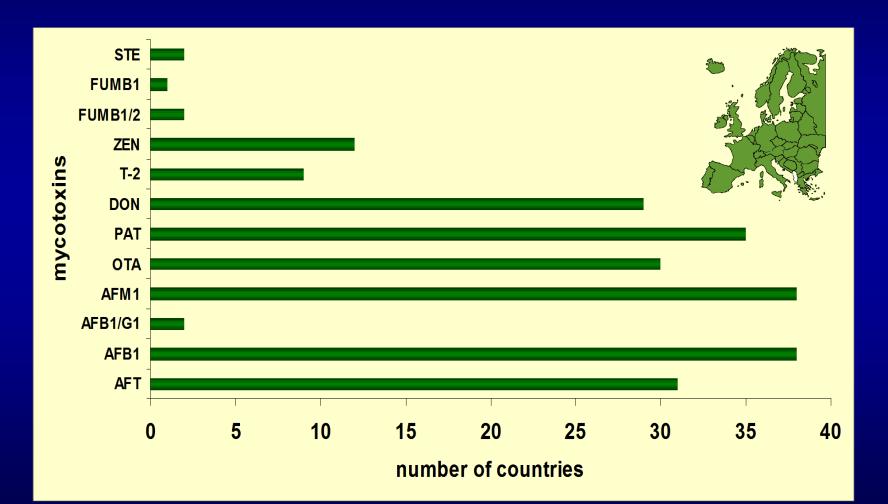


Mycotoxin regulating countries in Europe (FAO FNP 81, 2004)

- 39 nations with <u>known</u> 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)



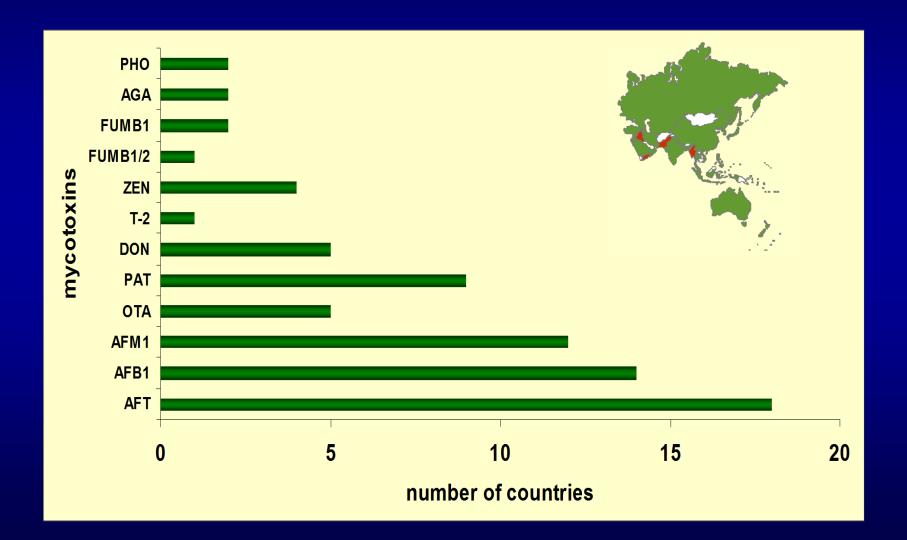
Mycotoxin regulating countries in Asia/Oceania

(FAO FNP 81, 2004)

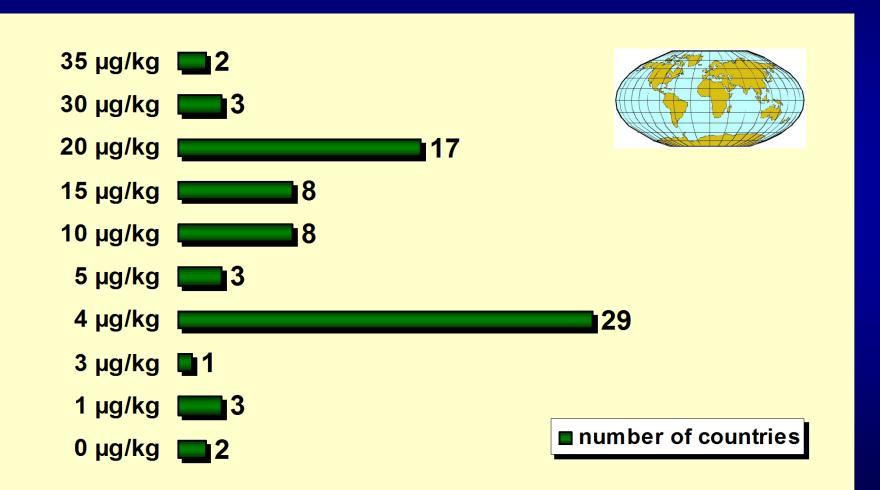
- 26 nations with <u>known</u> regulations (89% of inhabitants of the region)
- Regulations for <u>total</u> aflatoxins dominate in food, regulations for AFB₁ dominate in feed
- Harmonized regulations in Australia & New Zealand
- Most detailed : China and Iran



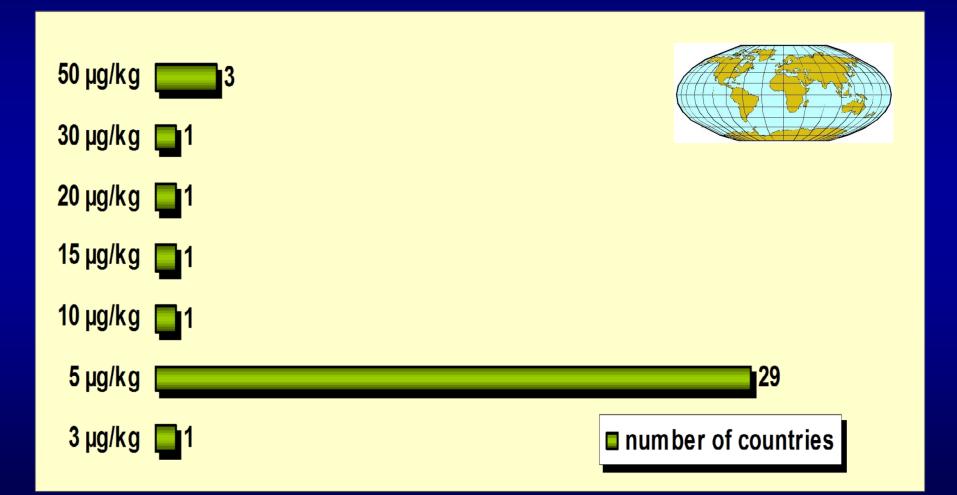
Asia/Oceania: mycotoxins regulated in food (FAO FNP 81)



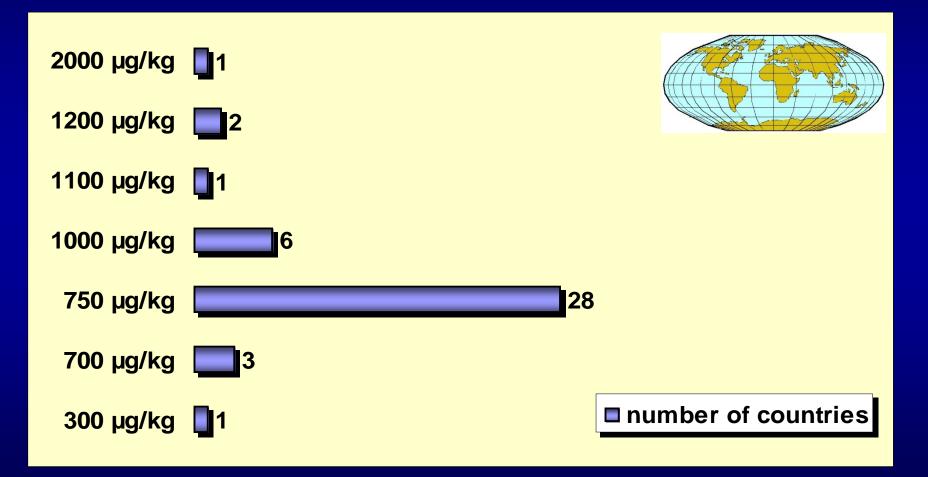
Total aflatoxins in food (FAO FNP 81, 2004)



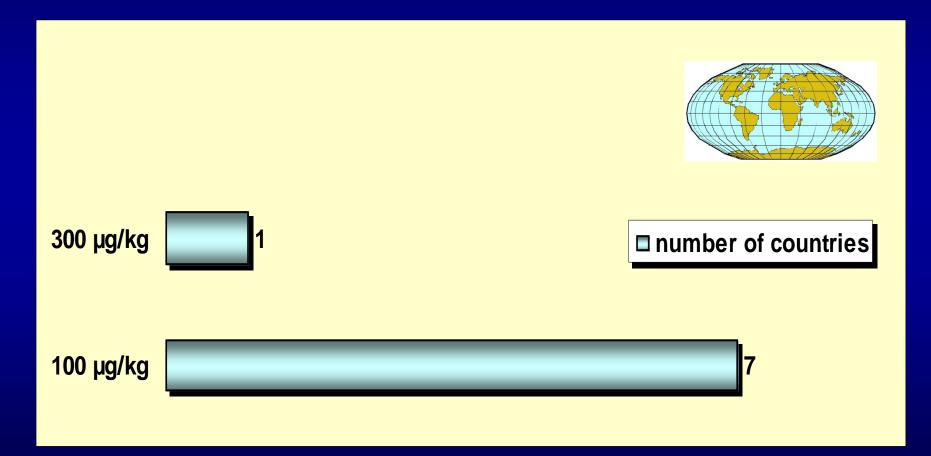
Ochratoxin A in cereals and cereal products (FAO FNP 81, 2004)



DON in wheat (flour) and other cereals (FAO FNP 81, 2004)



T-2 toxin in cereals and cereal flours (FAO FNP 81, 2004)









Antonio F. Logrieco

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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 (traceabilty)
 - Environmental
 - -CoC (Chain of Custoday)
 - Social Metrics Fair Trade, Fair Choice and GRASP
- Kabul Office Opened in 2014







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.



1111

WHO is your buyer? WHAT Does Your Buyer WANT?



Certification

- Certifications are ALL commercial standards
- Third-party certification = VERFICATION
- 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 Certficiation

- 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







1000 SAFETY









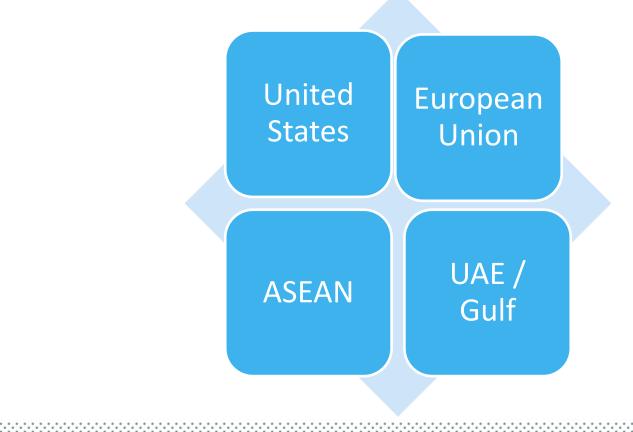
Why is ACCREDITATION important?

- 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



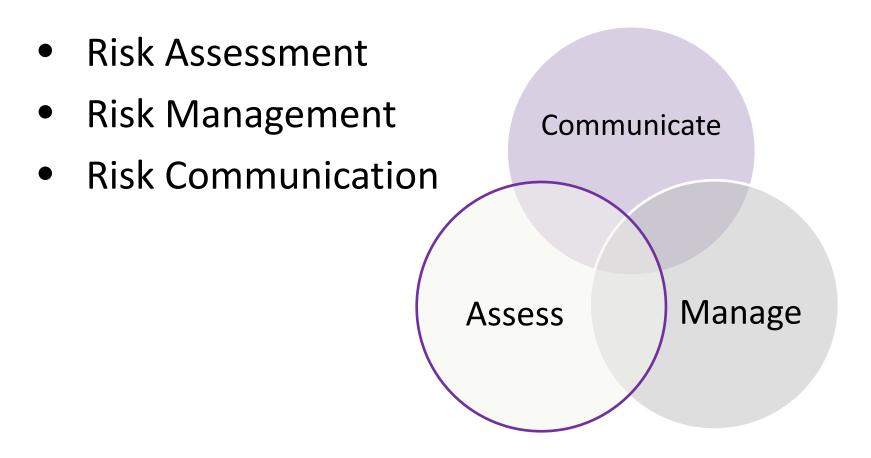


Risk Communications





Risk Communications as part of Risk Analysis







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





Elements of Risk Communications

- Share responsibilities coalition
- Know your audiences
- Be a credible source





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





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





Risk Communications Action Planning

- Interactive process
- Exchange of Information Opinion
- Concerning Risk
 - Risk-related factors Risk perceptions
- Throughout the Risk Analysis process





Risk Communications Action Planning

Among **Risk assessors Risk managers** Consumers Industry Academics, and others Includes **Risk assessment findings** Basis of risk management decisions



azaro

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Four Kinds of Risk Communications

Public Relations/ Precaution Advocacy Crisis Communications

Stakeholder Relations

> Outrage Management







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**



International Conference on Food Quality and Safety New Delhi, 14-15 March 2016

Pre-Harvest Mycotoxin Control

Ranajit Bandyopadhyay IITA, Ibadan, Nigeria

Acknowledgement Alejandro Ortega-Beltran Themis Michailides, UC Davis, Kearney

A member of CGIAR consortium

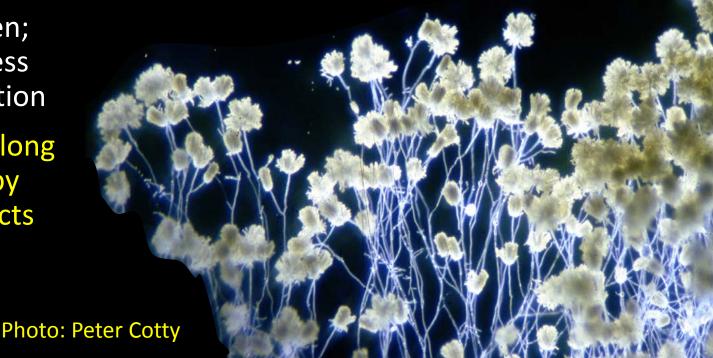
Agriculture for Nutrition & Health



Aflatoxin Facts

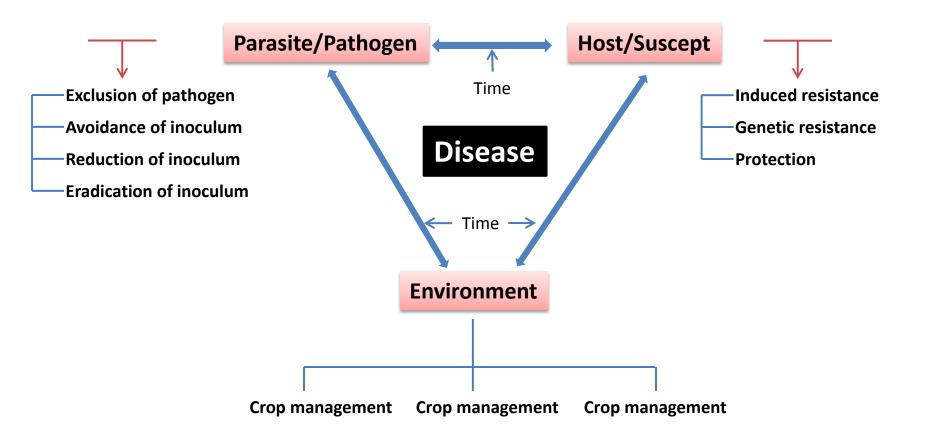
- Highly toxic metabolite produced by the ubiquitous Aspergillus Section Flavi
- The fungus resides in soil and crop debris, infects crops and produces the toxin in the field and in stores
- Weak pathogen; injury and stress increase infection
- Survives for a long time; spread by wind and insects

 Climate change increasing incidence and severity of aflatoxins





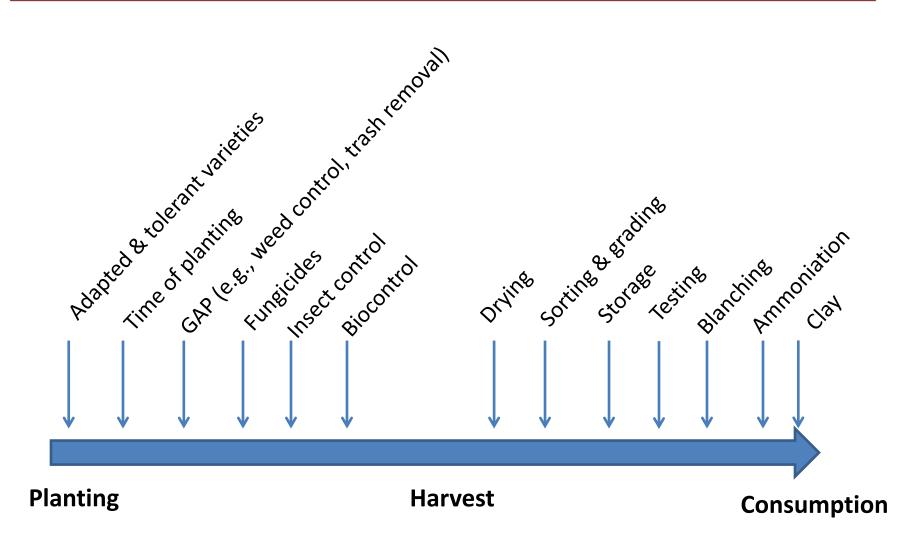
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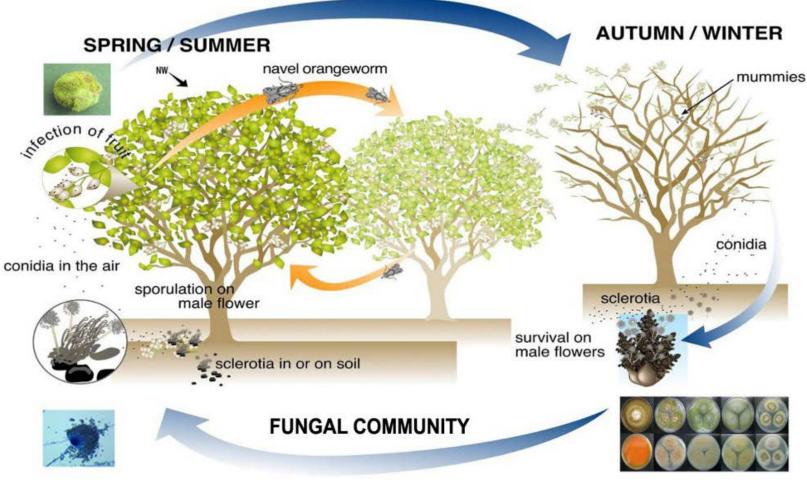
Agriculture for Nutrition & Health

Multiple practices to Manage Mycotoxins





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Source: http://dx.doi.org/10.5772/45918 2

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Agriculture for Nutrition & Health



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/

Agriculture for Nutrition & Health



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

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Agriculture for Nutrition & Health



Winter sanitation for navel orange worm control in almond and pistachio orchards



Decreasing the number of nuts left after harvest will reduce overwintering navel orange worm/*Aspergillus flavus* populations

Photo credit: http://almonds.com

A member of CGIAR consortium

Agriculture for Nutrition & Health







Application are done with the aid of Quad Motorcycles

Over 50% less aflatoxins result from AF36's applications

r noto creatt. http://azcotton.org/

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Agriculture for Nutrition & Health



- 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: <u>www.apsnet.org</u>

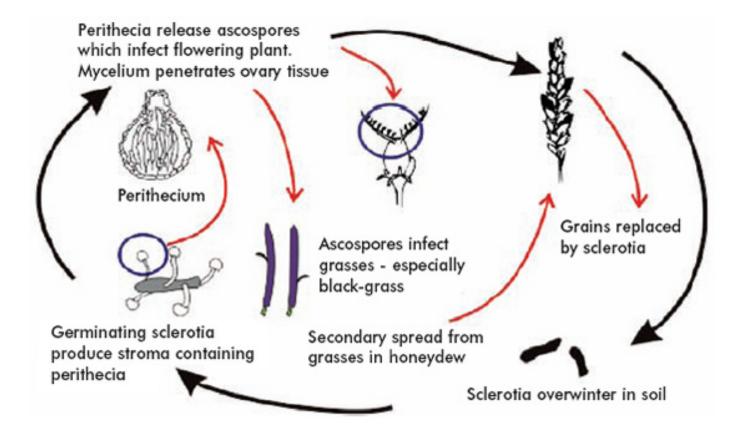
Wheat ergot

- 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



Source: http://cereals.ahdb.org.uk/

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 selfpollinated 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



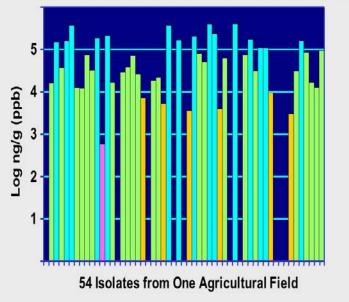
Source: University of Nebraska



Biocontrol Principles

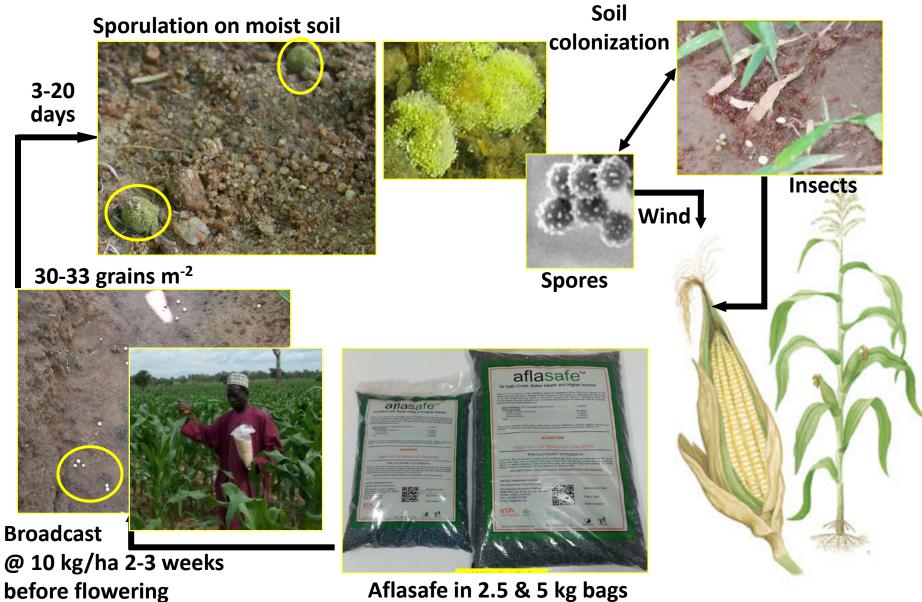
- In nature, some strains produce a lot (toxigenic), and others no aflatoxin (atoxigenic)
- Atoxigenic strains are already present on the crop
- Increase the frequency of atoxigenic strains to competitively displace toxigenic strains to reduce aflatoxin contamination
- Atoxigenic strains can be applied 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

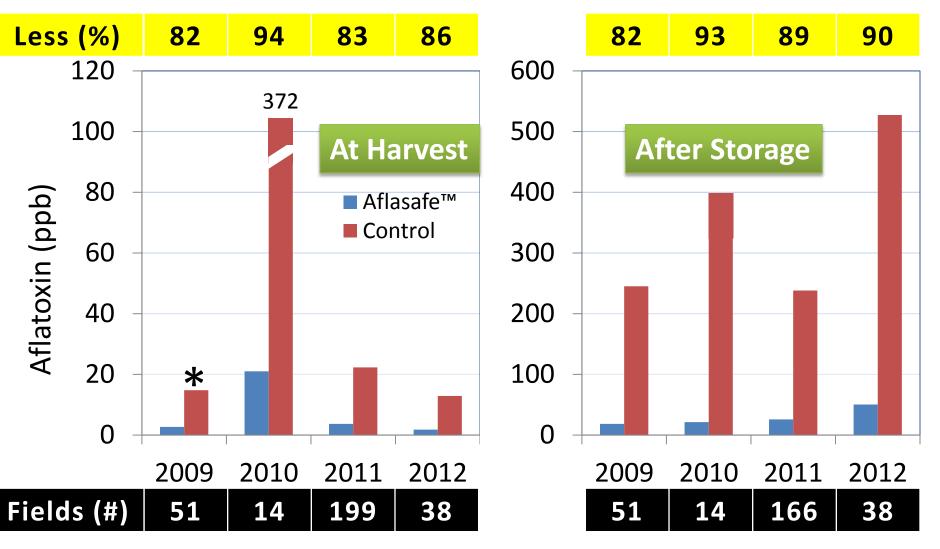




Aflasafe in 2.5 & 5 kg bags



Nigeria: Efficacy on Maize



*All means of aflasafe and control pairs significantly different; Student's t-test (P<0.05)



This Manufacturing Facility in IITA-Ibadan can supply aflasafe to treat 2 million ha annually





A member of CGIAR consortium

Agriculture for Nutrition & Health

www.iita.org



CGIAR

Aflasafe benefits Smallholder farmers

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%	Grain lots meet
Samples with <10 ppb AF (%)	99.5%	96%	international
samples with < 20 ppb AF (%)	99.5%	98%	standards
Return on Investment (ROI)	210%	489%	Higher income
Average sale price over market rate	13%	17%	
Aflasafe maize kept for family	46%	20.3%	Better health

Smallholder farmers have safer crops, improved income and better health

A member of CGIAR consortium



Aflasafe helps Kenya food security project

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







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

<u>Food Safety</u> 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

<u>Food Quality</u> 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
- <u>Chemical</u>: heavy metals, natural toxins, sanitizers, pesticides, antibiotics
- **<u>Physical</u>**: 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

- <u>Chemical hazard</u>: 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

<u>Physical hazard</u>: 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 <u>PREVENTION</u> 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

Hazard Categories			
1	2	3	4
Catastrophic	Critical	Serious	Minor
1A	2A	ЗA	4A
1B	2B	3B	4B
1C	2C	3C	4C
1D	2D	3D	4D
1E	2E	3E	4E
	1A 1B 1C 1D	1 2 Catastrophic Critical 1A 2A 1B 2B 1C 2C 1D 2D	123CatastrophicCriticalSerious1A2A3A1B2B3B1C2C3C1D2D3D



Unacceptable

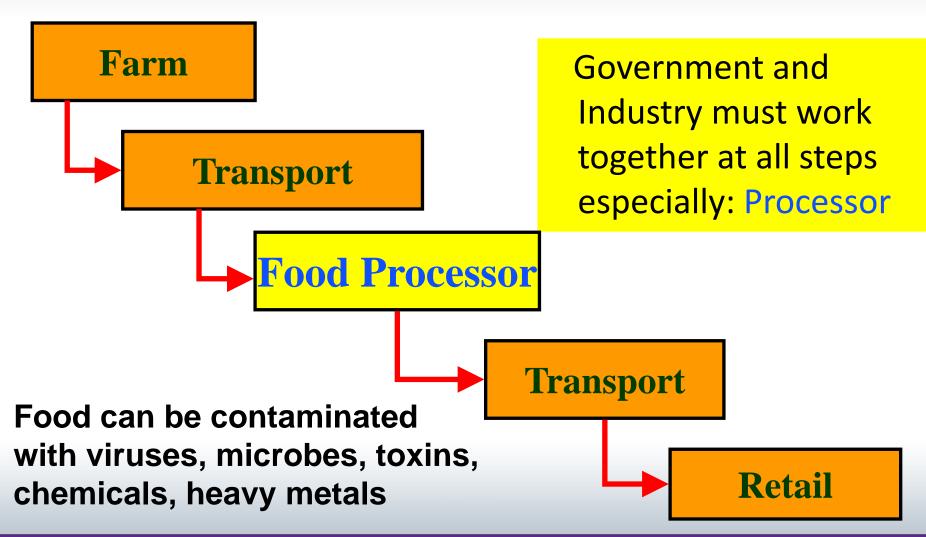
High

Medium

Low

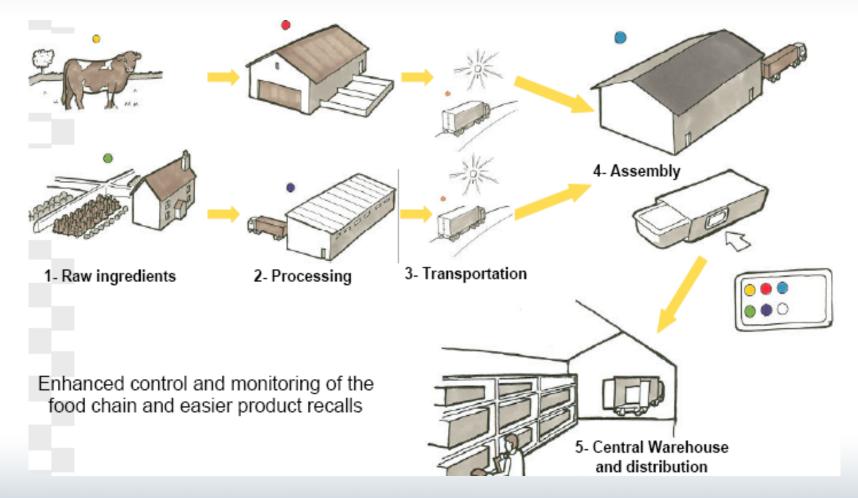


Issues Impacting Food Safety



KANSAS STATE College of Agriculture

Food Safety and Traceability





SAFE FOOD FOR ALL

SHARED RESPONSIBILTY

Food Legislation and Enforcement

Advice for Industry/Trade

> Consumer Education

Information Gathering and Research

Provision of Health-Related Services

GOVERNMENT

Educated and Knowledgeable Public

Discriminating and Selective Consumers

Safe Food Practices in the Home

Community Participation

Active Consumer Groups

CONSUMER

Good Practices by Primary Producers and Distributors

Quality Assurance and Control of Processed Food

Appropriate Processes and Technology

Trained Managers and Food Handler

> Informative Labeling and Consumer Education

INDUSTRY/TRADE

WHO Leadership for International Consensus on Food Safety Issues, Policies, and Actions

Additional Resources

- Centers for Disease Control and Prevention

 <u>http://www.cdc.gov/foodsafety/</u>
- U.S. Department of Agriculture
 - <u>http://www.foodsafety.gov</u>
 - <u>http://fnic.nal.usda.gov/about-fnic</u>
- Food and Drug Administration (FDA)
 <u>http://www.fda.gov/Food/</u>









College of Agriculture

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?
 - Area?
 - 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
 - HAACP 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 nonproducers.
- 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 <u>one</u> 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.

ImageIncreaseProvidencePro		Ci	ity	L	ake	Mou	ntain	Oc	ean	Va	lley	Desmanae
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								3	13			Funds from non-governmental sources

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	<i>x</i> #	^y S	#	S	#	S	#	S	#	S	Response
28									3	11	Technical training throughout the value
20										11	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. employ-
52									2	10	ees
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	٠	٠									Train staff in equipment use
39	•	•									Train staff in data validation
40	•	•									Trend analyses
41			-								Management capacity to prepare plans and
41			•	•							analyze results
42					•	•					Weather data from growing areas
43					•	•					Distribution of results from current survey
44							٠	٠			Central lab in Kabul
45							٠	٠			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
											Practical application of theoretical
50									•	•	knowledge and skills

Identify data that should be collected to enable decisions regarding mycotoxin contamination to be made in Afghanistan.

	Ci	ity	La	ake	Mou	ıntain	D
	<i>x</i> #	^y S	#	S	#	S	Response
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 in- dicators
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 vis a vis 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
13			Z	5			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	$\frac{2}{2}$	3					Healthy seed
	2	5					Public Trade Law Enforcement Actions and track reports of
19			2	3			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	•	٠					Mycotoxin levels pre-harvest with supporting samples
25	•	•					Mycotoxin levels in stored products
26	•	•					Moisture content measurements
27	•	•					Data trends

Identify ways to increase the credibility of the results obtained from mycotoxin surveillance surveys in Afghanistan.

	Mou	ntain	Oc	ean	Va	lley	Desmanae
	<i>x</i> #	^y S	#	S	#	S	Response
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
4			5	9	4	11	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 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

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

	C	ity	Va	lley	
	<i>x</i> #	^y S	#	S	Response
1	6	28	5	21	Define roles and responsibilities for each ministry
2	2	6	6	29	Interministirial/private sector task force with regular meetings and infor- mation 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		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	٠	•	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
22	1	1			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	٠	٠			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

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	<u>x</u> #	ike ^y S		<u>intain</u> S	<u> </u>	cean S	Response
- 1							
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	•	٠	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	٠	•			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 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	٠	•					Annual review of progress
34	•	•					Authorized testing program
35	•	•					Web site on regulations and how to meet them

Identify regulations needed to limit mycotoxin exposure in Afghanistan.

Identify cultural barriers to be overcome to reduce exposure to mycotoxins in Afghanistan.

	Mou	ntain	Va	lley	D
	<i>x</i> #	^y S	#	Š	Response
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	+	11	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	5)	3	9	Acknowledging the risk equals the word "dirty"
13			3	4	Food labeling
14	2	7	5		Limited cooperation among farmers and between associations
15	$\frac{1}{2}$	5			Mycotoxin risk will be new and unknown
16	-	U	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

	C	ity	L	ake	Va	lley	D
	<i>x</i> #	^y S	#	S	#	Š	Response
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

Identify benefits resulting from lesser exposure to mycotoxins in Afghanistan.

Question 8-1A

	La	ake	Oc	cean	Va	lley	Pagnongo
	<i>x</i> #	^y S	#	S	#	S	Response
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, e.g., customs
5	4	15	٠	•	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	٠	•			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

Who needs information on mycotoxins in Afghanistan?

Question 8-1B

	La	ıke	Oc	ean	Va	lley	Response
	<i>x</i> #	^y S	#	S	#	S	Kesponse
1	1	1	8	40	4	19	Public media (radio, TV, print, etc.)
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 associa-
10	0	15					tions
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			٠	•			Farmer to farmer
23					•	٠	Comic books & posters
24					•	•	Mobile (traveling) theater

How should information on mycotoxins in Afghanistan be delivered?

Question 8-2A

	C	ity	Mou	Intain	Perpanse
	<i>x</i> #	^y S	#	S	Response
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

When should screening for mycotoxins occur in Afghanistan?

Question 8-2B

	C	ity	Moi	ıntain	Desponse
	<i>x</i> #	^y S	#	S	Response
1	8	34	6	18	Preharvest – in the field
23	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

Where should screening for mycotoxins occur in Afghanistan?

Question 11-1A

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

	City		La	ake	Mountain		Ocean		Va	lley	D
	<i>x</i> #	^y S	#	S	#	S	#	S	#	S	Response
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	٠	٠					7	38			Establish mycotoxin regulations
11	2	14	4	18							Form consumer protection group
											Identify MAIL/Commerce/Public
12	2	7							3	14	Health/Private sector roles and responsibil- ities
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 contami- nation
16	•	•					3	10			Develop and implement Good Agricultural
							-	-			Practices (post-harvest)
17	•	٠					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 HAACP baselines
22			5	17							Training for trade association members
23			5	12							Sensitize staff at all three ministries to the
24							4	22			problem Identify stakeholders
24							4				Identity stakenoluers

	Ci	City		ıke	Mountain		Ocean		Valley		Degnonge
	<i>x</i> #	^y S	#	S	#	S	#	S	#	S	Response
25	4	21									Infrastructure development
26							4	10			Improve knowledge sharing amongst stakeholders
			_								Adapt manuals from California Pistachio
27			3	18							Association for local use
28	3	12									Policy development
29	2	8									Implementation plan and budget by minis-
29	Z	0									try
30			2	7							Laboratory manual with SOPs
31									1	7	Private sector trade missions to Afghani-
51									1	/	stan
32					1	5					Technical assistance for farmers
33	1	1									Implement mycotoxin control program
34	•	٠									Send samples abroad for testing
35	•	٠									Develop regulatory mechanisms
36							•	•			Identify products to use to reduce myco-
50							•	•			toxin contamination
37									٠	•	Local training for certification

Question 11-1B

	City		Lake		Mountain		Ocean		Valley		D
	<i>x</i> #	<i>yS</i>	#	S	#	S	#	S	#	Š	Response
1			(20	_	_	7	20	2	10	Accreditation for labs (public and private
1			6	30	•	•	7	20	3	10	sector)
2	2	5	4	22			4	20	•	•	Human and Institutional Capacity Devel-
2	2	5	-				-	20	•	•	opment (HICD)
3	•	•			2	8	4	11			Develop and implement Good Agricultural
	4	01					7				Practices – pre-harvest & post-harvest
45	4 3	21 7					7 8	30 42			Improve infrastructure (labs)
5	3	/									Develop mycotoxin mitigation strategies Adopt standards for maximum contamina-
6					2	8	9	37			tion allowed
7	1	5					9	44			Establish regional labs for surveillance
8	4	14	4	22							Government funding
9			5	23					3	13	Design and implement food safety
			5	23					5	15	(HAACP) interventions
10					5	12	3	8			Establish food safety authority
11	5	18							•	•	Local training for certification
12	•	•	4	22							Sustainable system for oversight and en-
13		•							3	16	forcement Retter testing/decumentation for exports
13	•	•					6	21	5	10	Better testing/documentation for exports Establish national policies
15			6	20			0	21			Modules/courses in degree programs
16			6	16							SOPs for all toxins along value chains
											National surveillance and data collection
17			5	17							program
18			4	22							Training for research & extension staff
19							4	15			Identify alternative uses/markets for con-
		1.4					•	10			taminated products
20	4	14					4	14			Donor funding
21							4	14			Strengthen border control/quarantine
22							3	16			Establish independent legal enforcement body
											MAIL/Commerce/Private sector working
23									3	10	group
24									3	9	Public awareness campaign(s)
25					3	4					Organize/strengthen farmer's cooperatives
26	2	14									Communication strategy
27									1	7	Promote healthy diets and food choices
28									1	3	MAIL/Ministry of Public Health monitor-

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

	City		La	ıke	Mou	ntain	Oc	ean	Valley		Degnongo
	<i>x</i> #	^y S	#	S	#	S	#	S	#	S	Response
											ing strategy
29									1	3	Implementable control strategy from inter-
											ministerial/private sector collaborations

Notes:

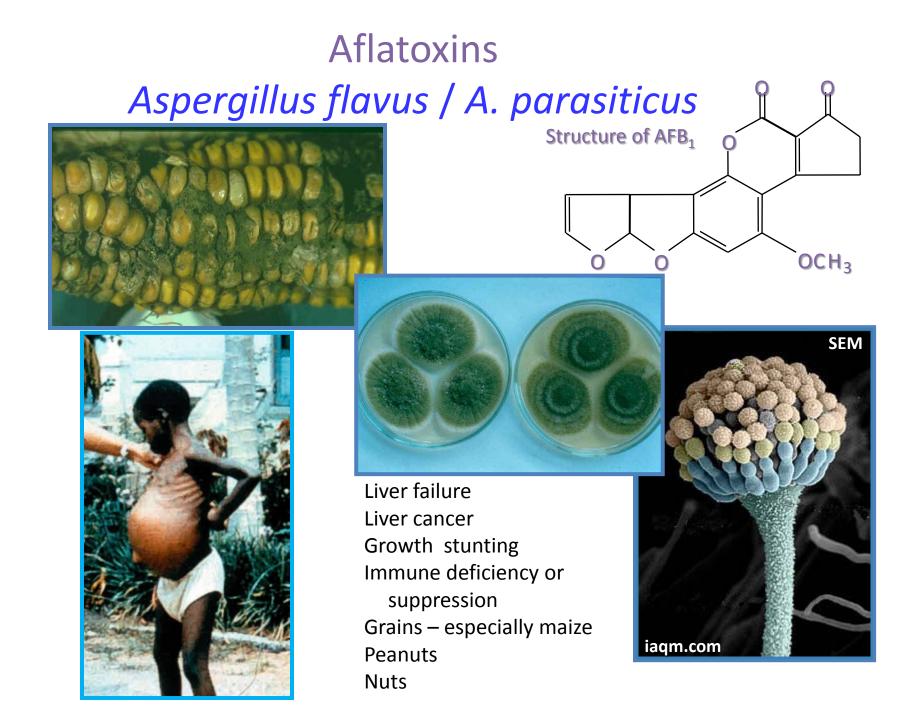
- $x^{\#}$ Number of persons ranking this response as one of the five (seven, for questions 11-1A and 11-1B) most important.
- ${}^{y}S$ Weighted priority score, with each voting member ranking their top five topics. Five points assigned to the most important response and one point to the least significant 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



Ochratoxins Aspergillus ochraceus

Kidney failure

Cacao

Grapes

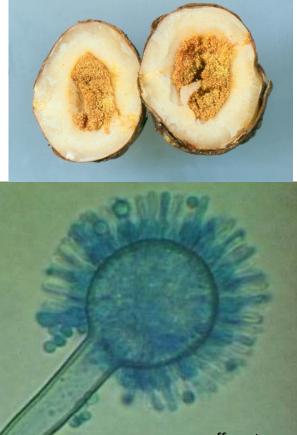
Coffee

Wheat

Nuts





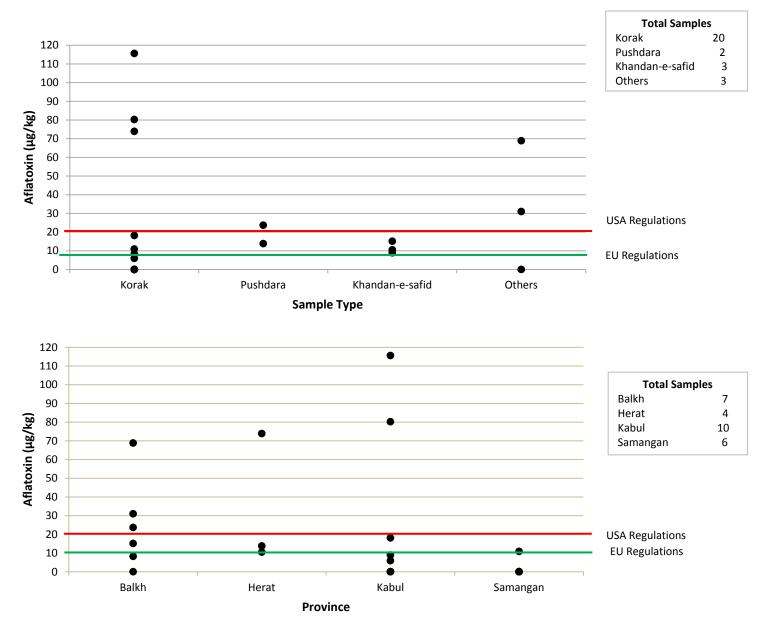


coffee-ota.org

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

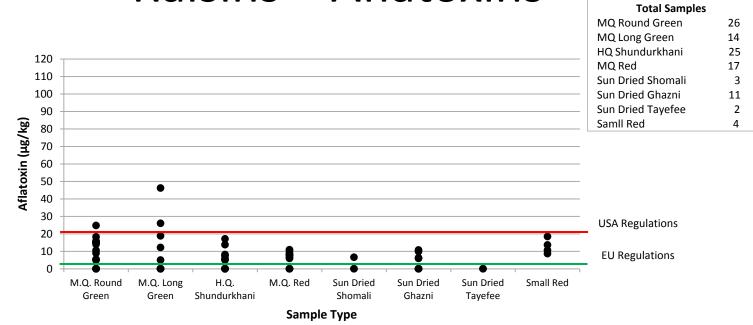
Pistachios – Aflatoxins

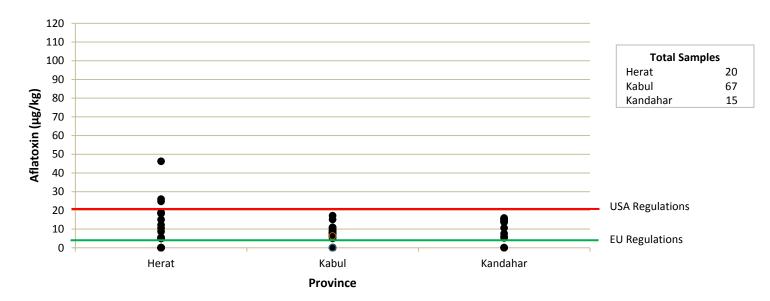


Raisins – Results

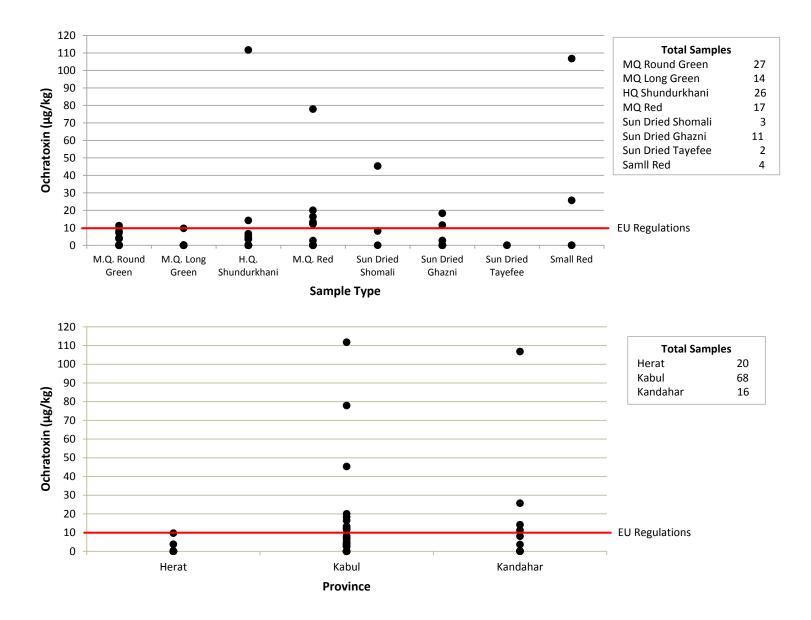
- Aflatoxins could limit exports in 43/89 samples
- Ochratoxin could limit exports in 25/80 samples
- Raisin type and drying method can be important
- Afghanistan and Austria results are discordant
- Choice of country to export to may depend on level of contamination

Raisins – Aflatoxins





Raisins – Ochratoxin



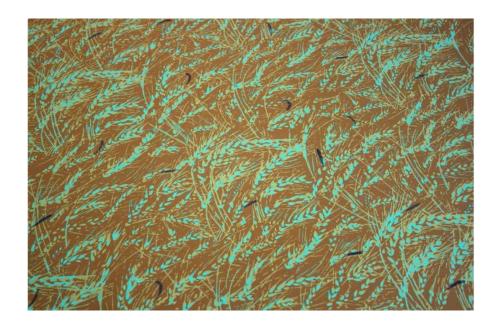
Trichothecenes

- 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

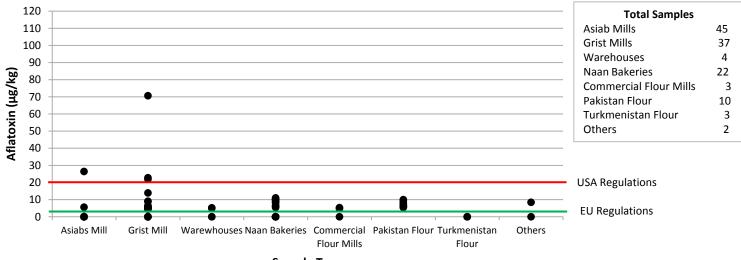
- 945 France
- 1093 France
- 1692 USA
- 1926 Russia
- 1929 Ireland
- 1953 France
- 1958 India
- 1973 Ethiopia



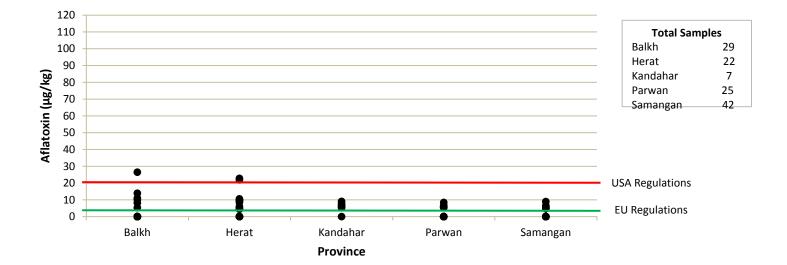
Wheat – Results

- 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

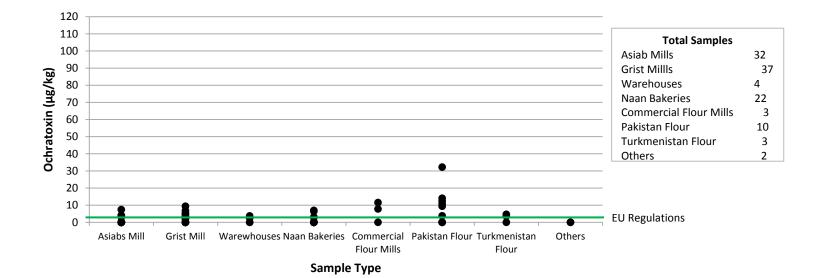
Wheat - Aflatoxin

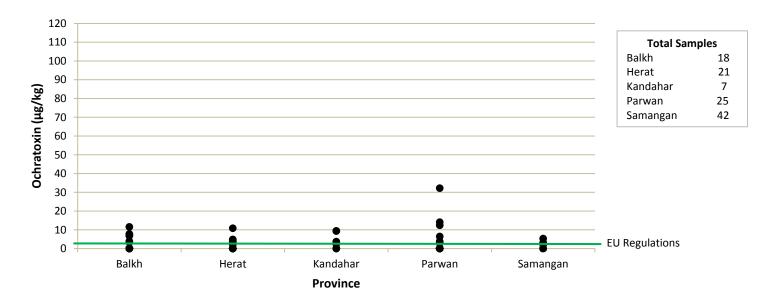


Sample Type



Wheat – Ochratoxin





Austrian Screen – Wheat

Fusarium	Alternaria	Aspergillus	Penicillium	Claviceps
Beauvericin	Alternariol	Aflatoxin	Agroclavine	Ergocristine
	Alternariol			
Enniatin A	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
		Methoxysterigm	Mycophenolic	
Epiequisetin	Macrosporin	atocystin	acid	Ergosinin
		3-Nitropropionic		
Equisetin	Tentoxin	acid	Questiomycin A	Ergotamine
	Tenuazonic		Quinolactacin	
HT-2 toxin	acid	Norsolorinic acid	А	Ergotaminine
			Secalonic acid	
T-2 toxin		Ochratoxin	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
 - HAACP 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	Sample ID	Afghanistan	atoxin	Afghanistan	1
Number	Sample ID	Romer	Austria	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
310 311	R02-H-S2-8-12-15-015-005	5	< LOD < LOD	< LOD < LOD	< LOD < LOD
311	R01-H-S3-8-9-15-013-006 R01-H-S3-8-12-2015-014-002	25	< LOD < LOD	< LOD < LOD	< LOD < LOD
312	R03-KBL-S1-4-10-15-344-022	< LOD	< LOD	5	< LOD < LOD
314	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
352 355	R01-KBL-S1-4-10-15-362-023 R03-KBL-S1-03-10-15-322-009	< LOD 8	< LOD < LOD	< LOD < LOD	< LOD < LOD
355	R04-KBL-S1-10-11-15-458-007	< LOD	< LOD < LOD	78	< LOD < LOD
361	R03-KBL-S1-10-3-15-361-015	< LOD < 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
391 392	R06-KBL-S1-10-10-15-434-023	< LOD < LOD	< LOD < LOD	3	< LOD < LOD
395	R01-KBL-S1-4-10-15-355-020 R03-KBL-S1-10-3-15-316-011	< LOD	< LOD < LOD	< LOD	< LOD < LOD
401	R06-KBL-S1-10-5-15-394-005	10	< LOD	< LOD	< LOD < LOD
401	R03-KN-S2-9-9-15-0212-001	10	< 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 11	< LOD < LOD	< LOD < LOD	< LOD
437 438	R04-KBL-S1-10-4-15-346-005 R04-KBL-S1-10-11-15-453-004	<pre>11 <lod< pre=""></lod<></pre>	< LOD < LOD	< LOD < LOD	< LOD < LOD
438	R04-KBL-S1-10-11-15-453-004 R01-KBL-S1-3-10-15-332-026	< LOD < LOD	< LOD < LOD	< LOD < LOD	< LOD < LOD
439	R03-KBL-S1-10-3-15-315-016	< LOD	< LOD < LOD	3	< LOD < LOD
441	R02-KBL-S1-10-3-13-313-010 R02-KBL-S1-10-11-2015-446-009	< LOD	< LOD	< LOD	< LOD < LOD
442	R02-KBL-S1-10-11-2015-449-012	Trace	< LOD	< LOD	< LOD
443	R06-KBL-S1-10-11-2013-443-012	< 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
			< LOD	< LOD	< LOD
454 458	R02-KBL-S1-10-10-2015-412-010 R01-KN-S2-9-9-15-214-011	< LOD 15	< LOD < LOD	11	< LOD < LOD

Aflatoxin < 4.0 > 4.0 - 10.0 > 10.0 - 20.0 > 20.0

Ochratoxin > 2.0 > 2.0 - 5.0 > 5.0 - 10.0 > 10.0

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

Random		Aflatoxin		Aflatoxin	
Random Number	Sample ID	Afghanistan	UNL	UNL	Austria
		Romer	Romer	Neogen	
462	A06-B-S4-8-31-15-015-008	< LOD	9	< LOD	< LOD
466	A03-B-S2-8-3-15-013-001	< LOD	< LOD	< LOD	< LOD
469	A07-B-S1-8-3-15-002-002	8	< LOD	< LOD	< LOD
470	A04-KBL-S1-10-3-15-318-0011	< LOD	< LOD	< LOD	< LOD
472	A01-KBL-S1-10-4-15-367-008	< LOD	146	109	13
473	A07-KBL-S1-10-3-15-305-010	< LOD	< LOD	< LOD	< LOD
474	A01-KBL-S1-10-4-15-378-009	< LOD	< LOD	< LOD	< LOD
476	A07-KBL-S1-10-4-15-347-008	< LOD	< LOD	< LOD	< LOD
477	A01-B-S1-8-3-15-010-003	8	< LOD	< LOD	< LOD
480	A07-KBL-S1-10-3-15-306-009	< LOD	< LOD	< LOD	< LOD
482	A04-KBL-S1-10-3-15-317-005	11	< LOD	< LOD	< LOD
483	A07-B-S2-8-3-15-020-005	Trace	6	< LOD	< LOD
484	A04-KBL-S1-10-3-15-307-009	Trace	< LOD	Trace	< LOD
485	A06-KBL-S1-10-4-15-341-017	< LOD	< LOD	< LOD	< LOD
487	A01-B-S2-8-3-15-045-006	11	549	224	3491
488	A03-H-S2-8-10-2015-0063-004	Trace	5	< LOD	6
489	A06-KN-S2-9-9-15-234-013	543	< LOD	< LOD	< LOD
490	A03-H-S4-8-12-2015-0084-003	< LOD	< LOD	6	< LOD
491	A01-KBL-S1-10-11-15-447-010	< LOD	< LOD	< LOD	< LOD
492	A04-B-S3-8-3-15-038-003	< LOD	< LOD	< LOD	1
493	A02-KBL-S1-10-10-15-435-004	6	< LOD	< LOD	< LOD
494	A07-KBL-S1-10-3-15-338-006	7	< LOD	< LOD	< LOD
496	A06-H-S4-8-7-15-062-002	< LOD	< LOD	< LOD	< LOD
497	A06-KBL-S1-10-3-15-336-017	Trace	< LOD	< LOD	< LOD
501	A04-KBL-S1-11-10-15-563-013	13	6	< LOD	< LOD
502	A07-KBL-S1-10-3-15-311-011	6	5	< LOD	< LOD
504	A04-KBL-S1-10-3-15-330-008	14	< LOD	< LOD	< LOD
510	A01-B-S4-8-3-15-004-002	22	< LOD	< LOD	< LOD
513	A04-B-S1-8-3-15-022-001	< LOD	< LOD	< LOD	< LOD
518	A06-KN-S1-9-9-15-230-016	< LOD	< LOD	< LOD	< LOD
519	A04-KBL-S1-10-3-15-337-010	Trace	< LOD	< LOD	< LOD
521	A03-H-S4-8-11-15-078-007	21	< LOD	< LOD	< LOD
522	A06-KBL-S1-10-4-15-383-012	Trace	< LOD	< LOD	< LOD
523	A02-B-S2-8-3-15-005-002	19	< LOD	< LOD	< LOD
524	A01-KBL-S1-10-3-15-309-004	< LOD	< LOD	< LOD	< LOD
525	A01-KBL-S1-10-3-15-308-011	< LOD	< LOD	< LOD	79
526	A01-KBL-S1-10-4-15-340-012	< LOD	< LOD	Trace	< LOD
527	A06-KBL-S1-10-4-15-354-015	< LOD	< LOD	< LOD	< LOD
528	A03-B-S1-8-03-15-021-005	158	167	96	208
529	A01-B-S1-8-3-15-023-001	8	< LOD	< LOD	< LOD
531	A04-KBL-S1-10-4-15-345-0012	< LOD	< LOD	< LOD	< LOD
533	A04-B-S2-8-3-15-044-004	13	< LOD	< LOD	< LOD
534	A04-B-52-8-3-15-004-004	< LOD	< LOD < LOD	< LOD	6
535	A03-B-S2-8-11-15-039-006	122	< LOD	< LOD	< LOD
536	A06-H-S5-8-10-15-060-009	< LOD	6	< LOD	< LOD
537	A01-KBL-S1-10-3-15-310-003	< LOD < LOD	< LOD	< LOD	< LOD
538	A03-B-S2-8-26-15-003-002	< LOD < LOD	< LOD < LOD	Trace	< LOD < LOD

Aflatoxin < 4.0 > 4.0 - 10.0 > 10.0 - 20.0 > 20.0

Random		Aflatoxin
Number	Sample ID	Afghanistan
Number		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		Aflatoxin		
fghanistan Romer	UNL Romer	UNL Neogen	Austria	
9	< LOD	< LOD	< LOD	Aflatoxin
116	12	13	1189	< 4.0
18	9	6	5	> 4.0 - 10.0
< LOD	< LOD	< LOD	1	> 10.0 - 20.0
< LOD	< LOD	20	< LOD	> 20.0
< LOD	< LOD	< LOD	< LOD	
< LOD	46	29	142	
11	< LOD	< LOD	< LOD	
8	< LOD	6	0.4	
184	< LOD	< LOD	1	
< LOD	100	95	14	
31	77	82	82	
< LOD	Trace	Trace	< LOD	
24	27	24	14]

Aflatoxin		
< 4.0		
> 4.0 - 10.0		
> 10.0 - 20.0		
> 20.0		

Develope		Aflatoxin		Aflatoxin	
Random Number	Sample ID	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

Random		Aflatoxin	Aflatoxin			
Number	Sample ID	Afghanistan	UNL	UNL	KSU	Austria
Number		Romer	Romer	Neogen	Romer	Austria
67	W02-PR-S1-10-29-15-533-024	Trace	5	< LOD	< LOD	< LOD
68	W06-S-S1-11-01-15-141-005	5	Trace	< LOD	< LOD	< LOD
71	W07-PR-S1-10-29-15-543-022	Trace	Trace	< LOD	< LOD	< LOD
72	W07-PR-S1-10-29-15-544-021	< LOD	Trace	< LOD	<lod< td=""><td>< LOD</td></lod<>	< LOD
73	W02-PR-S1-10-29-15-522-024	< LOD	Trace	< LOD	<lod< td=""><td>< LOD</td></lod<>	< LOD
74	W01-S-S1-10-29-15-032-031	Trace	Trace	< LOD	< LOD	< LOD
75	W07-PR-S1-10-29-15-540-023	< LOD	Trace	< LOD	<lod< td=""><td>< LOD</td></lod<>	< LOD
76	W02-S-S1-11-05-15-121-025	5	Trace	< LOD	< LOD	< LOD
77	W01-S-S1-10-28-15-012-037	< LOD	Trace	< LOD	< LOD	< LOD
78	W01-B-S2-9-19-15-093-020	< LOD	Trace	< LOD	< LOD	< LOD
79	W01-S-S1-10-29-15-036-043	Trace	Trace	< LOD	< LOD	< LOD
80	W11-PR-S1-10-29-15-541-003	8	Trace	< LOD	< LOD	0.50
81	W07-PR-S1-10-29-15-525-008	< LOD	Trace	< LOD	< LOD	< LOD
82	W07-PR-S1-10-29-15-546-011	< LOD	Trace	< LOD	< LOD	< LOD
83	W02-S-S2-11-01-15-133-017	< LOD	< LOD	< LOD	< LOD	< LOD
84	W07-PR-S1-10-29-15-530-009	< LOD	Trace	< LOD	< LOD < LOD	< LOD < LOD
85	W07-PR-S1-10-29-15-536-009 W02-PR-S1-10-29-15-536-029	5	<lod <lod<="" td=""><td><lod <lod<="" td=""><td><lod <lod<="" td=""><td>< LOD < LOD</td></lod></td></lod></td></lod>	<lod <lod<="" td=""><td><lod <lod<="" td=""><td>< LOD < LOD</td></lod></td></lod>	<lod <lod<="" td=""><td>< LOD < LOD</td></lod>	< LOD < LOD
87	W02-PR-S1-10-29-15-556-029 W01-B-S3-8-06-15-070-027	< LOD	< LOD / <lod< td=""><td>Trace</td><td><lod <lod<="" td=""><td>< LOD < LOD</td></lod></td></lod<>	Trace	<lod <lod<="" td=""><td>< LOD < LOD</td></lod>	< LOD < LOD
87	W01-B-53-8-06-15-070-027 W07-PR-S1-10-29-15-545-020	Trace	< LOD < LOD	< LOD	< LOD < LOD	< LOD < LOD
				-	-	-
91	W01-B-S2-8-3-15-081-025	< LOD	< LOD	< LOD	< LOD	< LOD
92	W07-PR-S1-10-29-15-547-010	< LOD	< LOD	< LOD	< LOD	< LOD
95	W01-B-S2-8-05-15-105-026	< LOD	< LOD	Trace	< LOD	< LOD
96	W07-PR-S1-10-29-15-538-015	< LOD	< LOD	< LOD	< LOD	< LOD
97	W02-PR-S1-10-29-15-532-028	Trace	< LOD	< LOD	< LOD	< LOD
98	W01-S-S2-10-29-15-028-046	Trace	< LOD	< LOD	< LOD	< LOD
100	W07-PR-S1-10-29-15-521-013	Trace	< LOD	< LOD	< LOD	< LOD
103	W07-PR-S1-10-29-15-520-014	< LOD	< LOD	< LOD	< LOD	< LOD
105	W01-S-S2-10-28-15-013-032	< LOD	< LOD	< LOD	< LOD	< LOD
106	W01-S-S1-10-28-15-010-034	< LOD	< LOD	< LOD	< LOD	< LOD
107	W01-S-S2-10-28-15-020-033	< LOD	< LOD	< LOD	< LOD	< LOD
110	W07-B-S2-8-4-15-063-001	10	< LOD	< LOD	< LOD	< LOD
111	W07-PR-S1-10-29-15-527-012	< LOD	Trace	< LOD	< LOD	< LOD
112	W02-S-S1-11-01-15-131-016	< LOD	< LOD	< LOD	< LOD	< LOD
114	W02-S-S1-11-01-15-105-026	Trace	< LOD	< LOD	< LOD	< LOD
115	W01-S-S1-10-29-15-026-044	Trace	< LOD	< LOD	< LOD	< LOD
116	W09-PR-S1-10-29-15-539-008	6	< LOD	< LOD	< LOD	< LOD
117	W09-PR-S1-10-29-15-523-010	6	<lod td="" trace<=""><td><lod <lod<="" td=""><td>< LOD</td><td>< LOD</td></lod></td></lod>	<lod <lod<="" td=""><td>< LOD</td><td>< LOD</td></lod>	< LOD	< LOD
118	W09-PR-S1-10-29-15-524-009	6	< LOD	< LOD	< LOD	< LOD
119	W07-PR-S1-10-29-15-529-022	5	5	< LOD	< LOD	< LOD
120	W09-PR-S1-10-29-15-528-006	7	< LOD	< LOD	< LOD	< LOD
121	W07-S-S2-11-1-15-143-021	6	< LOD	< LOD	< LOD	< LOD
122	W09-PR-S1-10-29-15-548-007	6	< LOD	< LOD	< LOD	< LOD
123	W02-S-S1-10-28-15-106-019	5	< LOD	Trace	< LOD	< LOD
126	W01-S-S1-10-28-15-001-036	< LOD	< LOD	< LOD	< LOD	< LOD
127	W02-S-S2-10-28-15-127-022	5	Trace	< LOD	< LOD	< LOD
128	W02-S-S1-10-28-15-137-017	Trace	Trace	< LOD	< LOD	< LOD
129	W02-S-S1-10-28-15-117-021	5	< LOD	< LOD	< LOD	< LOD
130	W02-S-S1-10-28-15-101-020	Trace	< LOD	< LOD	< LOD	< LOD
175	W02-S-S1-11-01-15-129-013	< LOD	< LOD	< LOD	< LOD	< LOD
180	W02-S-S1-11-01-15-135-015	< LOD	<lod <lod<="" td=""><td><lod <lod<="" td=""><td>< LOD</td><td>< LOD</td></lod></td></lod>	<lod <lod<="" td=""><td>< LOD</td><td>< LOD</td></lod>	< LOD	< LOD
183	W02-S-S1-10-28-15-125-018	< LOD	Trace	< LOD	< LOD	< LOD
185	W02-S-S1-11-01-15-113-014	< LOD	Trace	< LOD	< LOD	< LOD
184	W01-S-S2-10-28-15-024-040	< LOD	< LOD	< LOD < LOD	< LOD < LOD	< LOD < LOD
188	W01-3-32-10-28-13-024-040 W07-S-S2-11-1-15-142-023	7	6	< LOD < LOD	< LOD	< LOD
194	W07-S-S2-11-1-15-142-023 W09-PR-S1-10-29-15-549-011	5	5	< LOD < LOD	< LOD < LOD	< LOD < LOD
200			5	< LOD < LOD	< LOD < LOD	< LOD < LOD
200	W01-S-S2-10-28-15-023-038	Trace	5	< LOD	< LOD	< LOD



Random		DON
Number	Sample ID	Afghanistan
3	W01-B-S1-3-8-15-0087-007	8000 8000 8000 8000 8000 8000 8000 800
5	W01-B-S1-S-8-13-0087-007 W01-H-S3-8-10-15-043-005	< LOD
6	W01-H-S5-8-10-15-045-005 W07-H-S5-8-9-15-030-006	Trace
7	W07-H-55-8-9-15-030-006 W02-B-S3-8-4-15-069-002	
9		Trace 370
-	W01-H-S2-14-9-15-042-001	
10	W02-H-S3-8-10-15-040-004	Trace
11	W07-H-S1-9-9-15-206-002	600
14	W01-H-S2-8-10-15-046-004	1290
16	W02-H-S1-08-10-15-050-001	Trace
17	W02-H-S3-8-11-15-086-003	Trace
18	W07-B-S2-8-3-15-083-001	3625
19	W02-H-S3-08-10-15-051-002	< LOD
21	W01-B-S2-8-3-15-080-006	< LOD
23	W01-B-S3-9-14-15-066-021	Trace
24	W01-B-S3-8-4-2015-068-018	Trace
27	W01-B-S2-8-11-15-064-022	Trace
28	W01-B-S2-8-5-15-099-006	< LOD
30	W07-B-S3-8-3-15-084-003	Trace
32	W01-B-S3-8-5-2015-0103-016	Trace
35	W01-B-S4-8-3-15-088-028	Trace
36	W07-H-S3-8-9-15-028-007	Trace
39	W012-H-S5-8-9-15-025-001	Trace
40	W01-B-S3-8-04-15-065-023	Trace
41	W01-B-S1-8-5-15-095-010	< LOD
43	W08-B-S2-8-4-15-058-001	Trace
45	W01-B-S4-8-4-2015-067-017	Trace
47	W01-B-S2-8-3-15-091-015	360
48	W02-B-S2-8-3-15-082-001	Trace
51	W01-B-S2-8-3-15-0085-009	445
53	W01-B-S2-8-5-2015-097-018	Trace
57	W08-H-S2-8-11-15-070-003	Trace
59	W06-KN-S3-09-10-15-0245-002	< LOD
62	W06-KN-S3-09-10-15-0246-001	Trace
65	W012-H-S3-8-10-15-038-002	Trace
110	W07-B-S2-8-4-15-063-001	< LOD
111	W07-PR-S1-10-29-15-527-012	Trace
112	W02-S-S1-11-01-15-131-016	Trace
114	W02-S-S1-11-01-15-105-026	Trace
115	W01-S-S1-10-29-15-026-044	Trace
116	W09-PR-S1-10-29-15-539-008	Trace
117	W09-PR-S1-10-29-15-523-010	Trace
118	W09-PR-S1-10-29-15-524-009	Trace
119	W07-PR-S1-10-29-15-529-022	Trace
120	W09-PR-S1-10-29-15-528-006	Trace
121	W07-S-S2-11-1-15-143-021	Trace
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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 safetyspecific 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 base-line 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, 2016LOCATION: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

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
09:15 - 10:00	 Summary of Mycotoxin Assessment John Leslie, Kansas State University 	Director
10:00 - 10:15	Questions & Answers	
10:15 - 10:30	Tea Break	
10:30 – 11:30	 New Delhi Action Plan Review 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

16 July 2016

Time	Topics	Presenter/ Facilitator
12:30-13:30	Lunch	
13:45 – 14:30	 Risk Communication Plan/Work Group reports 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 Closing Remarks Group photo 	

Post-New Delhi Conference meeting Participants List

MAIL Planning & Policy General Directorate PQD RIA	Deputy Minister Acting Director General Planning	mir.haidari@mail.gov.af	707,899,870	passport	D0003249
PQD	5 5	shakir majaadi@gmajl.com			
. =-	Acting Director DDOD	snakn.majeeui@gman.com	700,182,623	MAIL ID Card	16098
RIA	Acting DirectorPPQD	iqbal.karimi@mail.gov.af	780,357,291	MAIL ID Card	15003
	Research Adviser ARIA	qudrat.soofizada@gmail.com	700,595,938	MAIL ID Card	16048
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PQD	Quarantine Inspection Officer	mrafirustami@yahoo.com	799,241,676	MAIL ID Card	15072
PQD	Quarantine Inspection Officer	jahedahady@gmail.com	700,275,706	MAIL ID Card	15075
1oPH	Director of Quality Control			MoPH HR GD	HRID NO: 36295
ЛоРН	Director of PND				
1oPH	Director of HIS				
1oPH	Director of Environmental Health				
1oPH	GD Preventive Medicine				
1oPH	Director of Health Promotion				
SAIN	Country Director				
1oCI	International Trade Dept		0700 224812/0 78 40 33 918	Tazkara #	31598
1oCI	International Trade Dept				
Vorld Bank					
AO	FAO Representative			UN Badge #	FAO-VIP002
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IK AID					
AO				Badge	FAO-CV00053
AO				Badge	FAO-NS00299
AO				Badge	FAO-CV00084
ansas State University					
BCMP	Communications Director	acosic@cbcmp.org	0795 62 68 36	Passport	A1271794
IAIL				MAIL ID Card	93
iosecurity Engagement Program US Embassy	Coordinator				
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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?	(This should help to summarize information received/acquired during the workshop).	High, medium or low priority	(When we should start /how long preparation is needed?)
Whom to ask?/How to double-check?	(This should help participants to identify/remember relevant institutions and sources of information).		
What are our objectives?/What we want to achieve communicating?	(This should help participants to define objectives of the communication campaign/activities).		
Whom we are talking to?/Who are our	Primary audience		
audiences?	Other audiences		
What are our messages?	Priority Message		
	Supporting message 1		
	Supporting message 2		
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?			





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?	(This should help to summarize information received/acquired during the workshop).	High, medium or low priority	(When we can start /how long preparation is needed?)
Whom to ask?/How to double-check?	(This should help participants to identify/remember relevant institutions and sources of information).		
What are our objectives?/What we want to achieve communicating?	(This should help participants to define objectives of the communication campaign/activities).		
Whom we are talking to?/Who are our	Primary audience		
audiences?	Other audiences		
What are our messages?	Priority Message		
	Supporting message 1		
	Supporting message 2		
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?			





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?	(This should help to summarize information received/acquired during the workshop).	High, medium or low priority	(When we can start /how long preparation is needed?)	
Whom to ask?/How to double-check?	(This should help participants to identify/remember relevant institutions and sources of information).			
What are our objectives?/What we want to achieve communicating?	(This should help participants to define objectives of the communication campaign/activities).			
Whom we are talking to?/Who are our	Primary audience			
audiences?	Other audiences			
What are our messages?	Priority Message			
	Supporting message 1			
	Supporting message 2			
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
 - o Fumonisins
 - Deoxynivalenol and other trichothecenes, *e.g.*, T-2
 - o Zearalenone
 - o 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 are Mycotoxins?

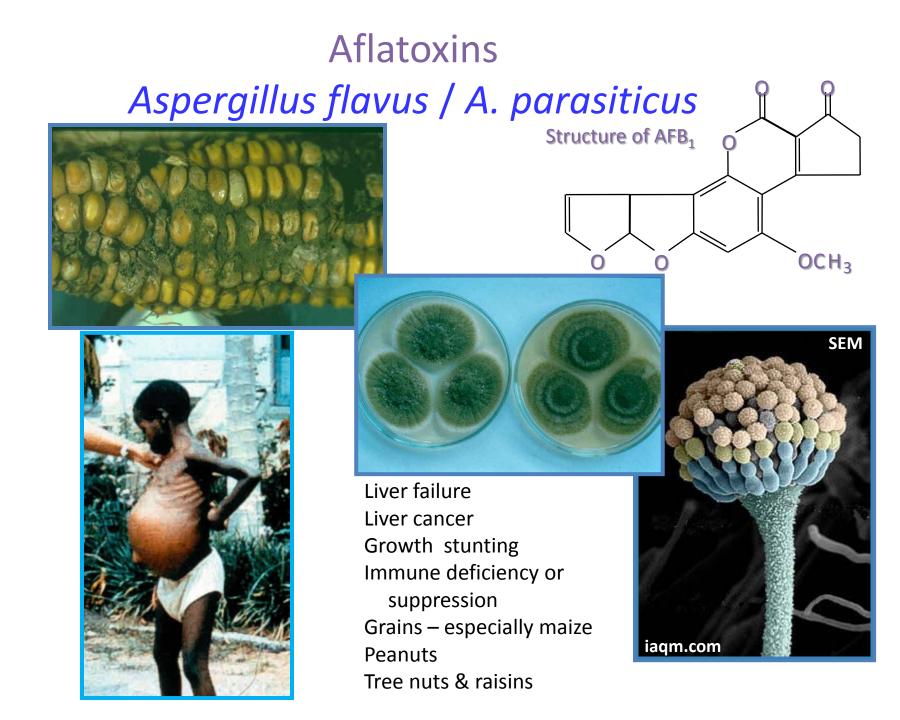
- Natural toxic metabolites produced by fungi
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 Aflatoxins
 - o Fumonisins
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 - o Zearalenone
 - **O Ochratoxin**
 - Ergot Alkaloids

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?

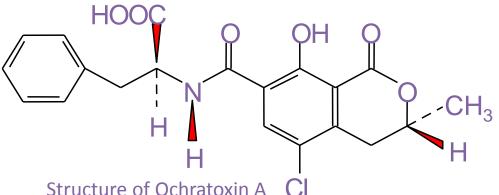
- Afghanistan MAIL and Deb Frey
 - Trained staff
 - Established and equipped a functional lab
 - Collected samples from across Afghanistan
 - Assayed raisins, nuts & wheat with Romer test kits
- Italy ISPA
 - UPLC and LC-MS assays for trichothecenes in wheat
 - Mycological analyses of flour
- Austria 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



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	16 10 0 2 2 11 R1
DON	–OH	-H	–OH	–OH	=O	9 11 2 3
3-ADON	–OAc	-H	–OH	–OH	=O	
15-ADON	–OH	-H	–OAc	–OH	=O	R5 ¹¹¹ 8 7 6 5 12 4
NIV	–OH	–OH	–OH	–OH	=O	R_{5} 7 5 4
T-2	–OH	–OAc	–OAc	-H	–OIsoval	R_{4}^{15} R_{2}^{15}
HT-2	–OH	–OH	–OAc	-H	–OIsoval	
4,15-DAS	–OH	–OAc	–OAc	-H	-H	R ₃

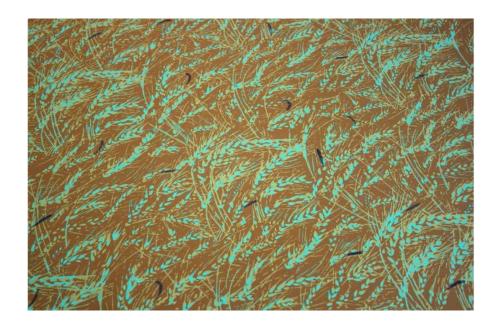


T-2 – Killed 1000s in Russia after WWII
DON – Becoming very widespread in US and Europe, especially where wheat and maize are grown
DON is changing the economic landscape of the US Great Plains *Fusarium* is the main producer on grains, but other fungi and some plants also synthesize

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



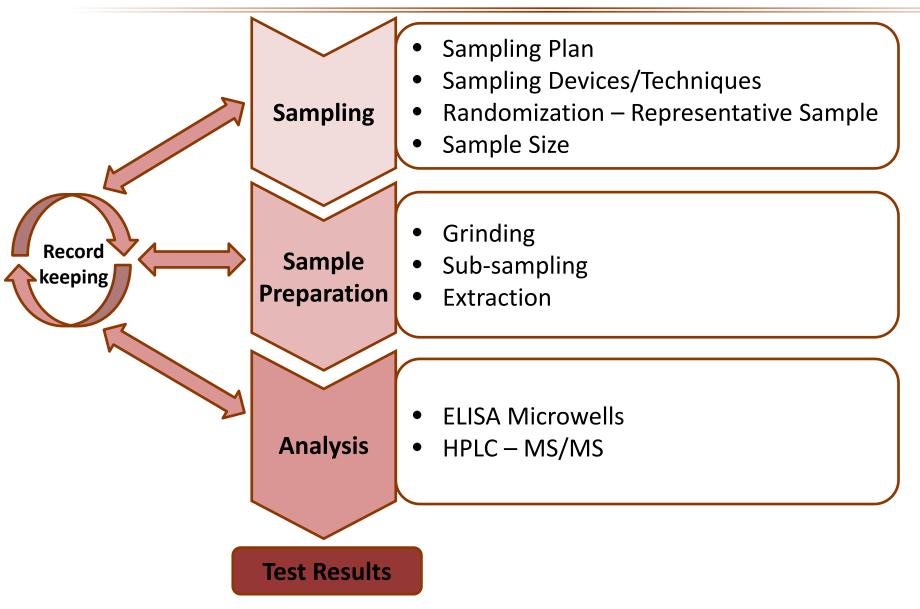


Some Ergot Epidemics -600 – Assyria 857 – Germany

- 945 France
- 1093 France
- 1692 USA
- 1926 Russia
- 1929 Ireland
- 1953 France
- 1958 India
- 1973 Ethiopia

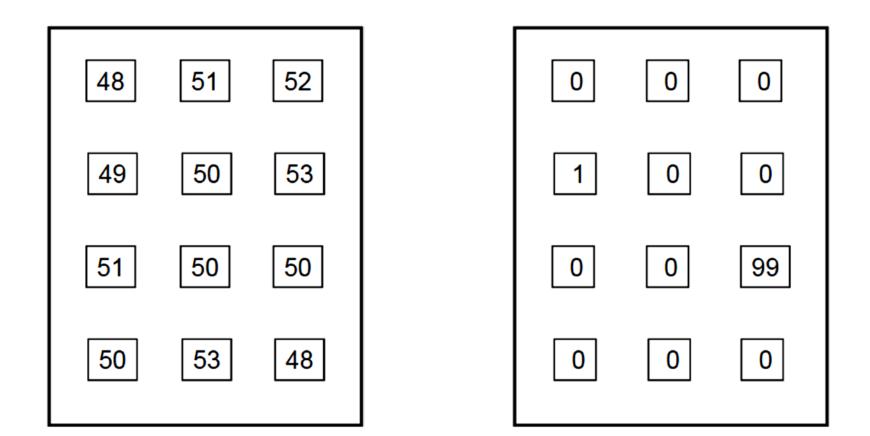


Mycotoxin Analysis – Main Steps





Sampling



Typical Protein Distribution

Typical Mycotoxin Distribution

Impact of Splitting Before Grinding

Mycotoxin	Commodity	Batch 1	Batch 2		
(µg/kg)	Commodity	Afghanistan	Austria	USA (UNL)	USA (KSU)
	Wheat-68	5	< LOD	< LOD	< LOD
Aflatoxin	Wheat-110	10	< LOD	< LOD	< LOD
Anatoxin	Walnut-551	25	< LOD	< LOD	
	Pistachio-624	< LOD	15	95	
Dooyuniyalanal	Wheat-3	3500	< LOD	< LOD	< LOD
Deoxynivalenol	Wheat-14	1290	< LOD	< LOD	< LOD
Ochratoxin	Raisin-296	13	< LOD		
	Raisin-302	< LOD	10		

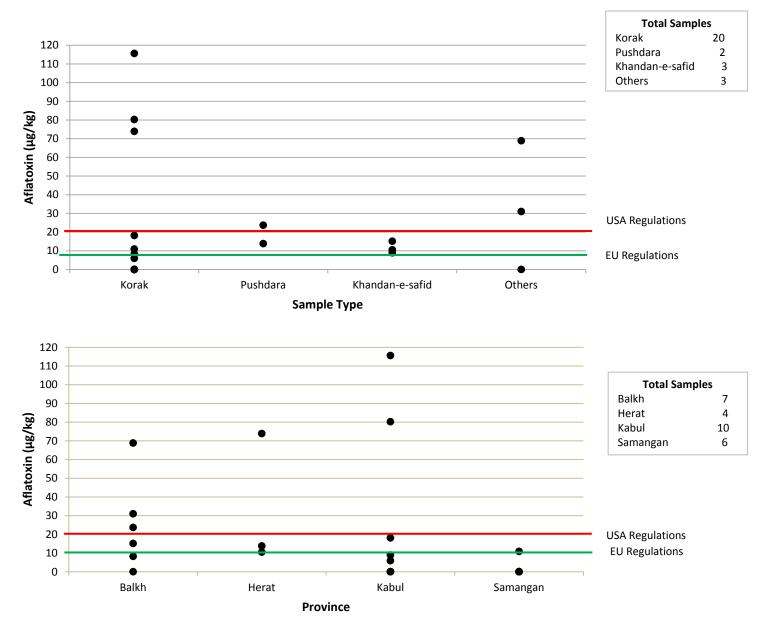
Test Kit Performance

- 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



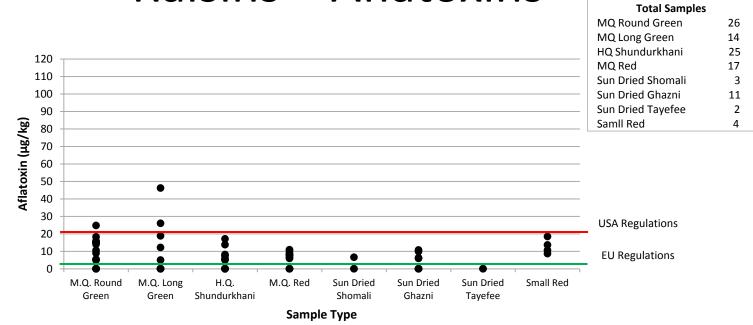
Austrian Screen – Nuts

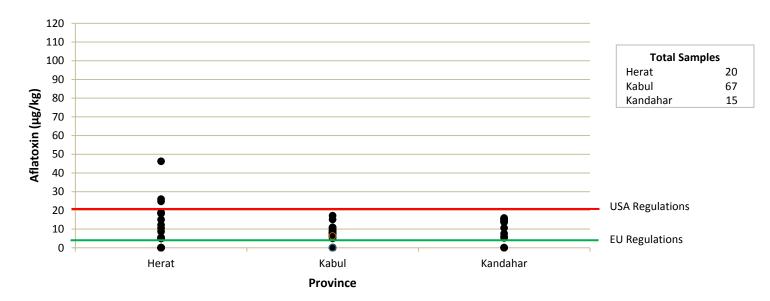
Fusarium	Alternaria	Aspergillus	Penicillium	
Butenolide	Alternariol	Cyclopiazonic Acid	Andrastin A	
	Alternariol methyl			
Epiequisetin	ether	Aflatoxin	Andrastin B	
Equisetin	Altersetin	Asperfuran	Agroclavine	
Fusaric acid	Infectopyron	Kojic acid	Chanoclavin	
HT-2 toxin	Macrosporin	Malformin A	Epoxyagroclavin	
T-2 toxin	Tentoxin	Malformin A2	Festuclavine	
Zearalenone	Tenuazonic acid	Malformin C	Mycophenolic acid	
			Mycophenolic acid	
α-Zearalenol		Nigragillin	IV	
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





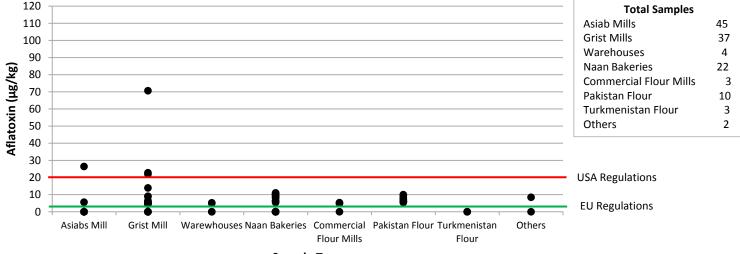
Austrian Screen – Raisins

Fusarium	Alternaria	Aspergillus	Penicillium
Fumonisins	Alternariol	Aflatoxin	Andrastin A
	Alternariol methyl ether	Aurasperon B	Andrastin B
	Altersetin	Aurasperon C	Andrastin C
	Altertoxin-I	Aurasperon G	Chanoclavin
	Macrosporin	Fonsecin	Festuclavine
	Tentoxin	Malformin A	Mycophenolic acid
	Tenuazonic acid	Malformin A2	Mycophenolic acid IV
		Malformin C	Penitrem A
		Nigragillin	Quinolactacin A
		Ochratoxin	
		Pyranonigrin	

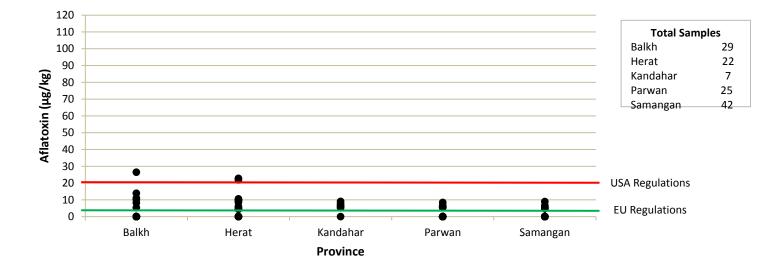
Wheat – Results

- 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

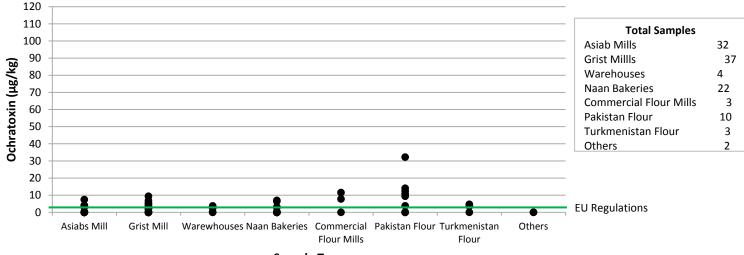
Wheat – Aflatoxin Afghan Diet Safety Limit – 0.14 µg/kg



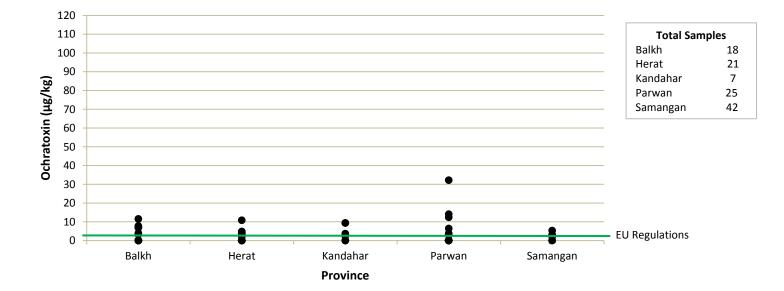
Sample Type



Wheat – Ochratoxin Afghan Diet Safety Limit – 0.7-9.8 μg/kg







Austrian Screen – Wheat

 \bigcirc

Fusarium	Alternaria	Aspergillus	Penicillium	Claviceps
Beauvericin	Alternariol	Aflatoxin	Agroclavine	Ergocristine
	Alternariol			
Enniatin A	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
		Methoxysterigm	Mycophenolic	
Epiequisetin	Macrosporin	atocystin	acid	Ergosinin
		3-Nitropropionic		
Equisetin	Tentoxin	acid	Questiomycin A	Ergotamine
	Tenuazonic		Quinolactacin	
HT-2 toxin	acid	Norsolorinic acid	А	Ergotaminine
			Secalonic acid	
T-2 toxin		Ochratoxin	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

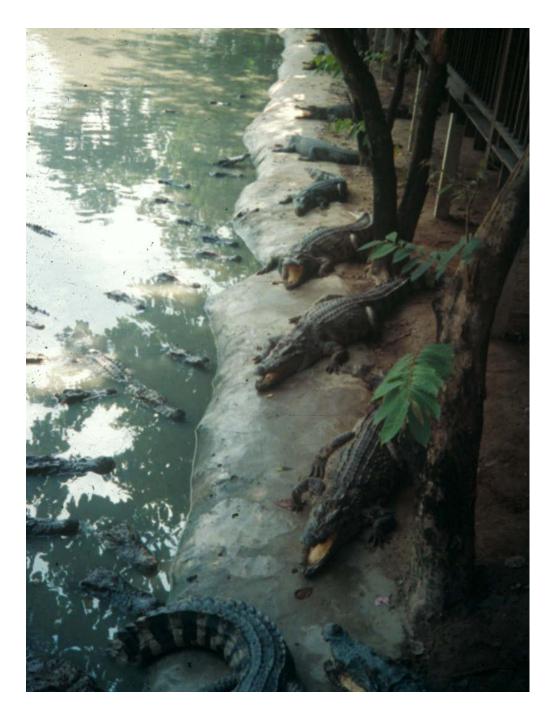


Special thanks to all of our collaborators in Afghanistan, Austria, Nebraska, Kansas (and my wife!).

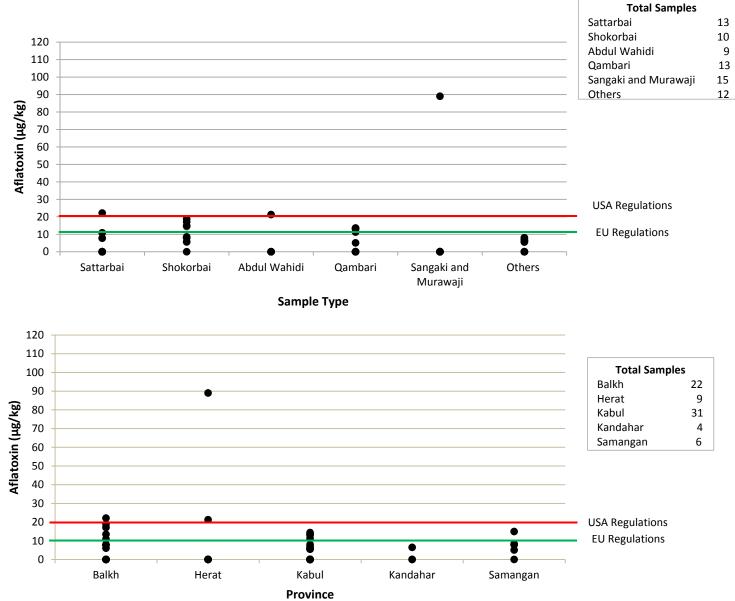
Questions?

"Where waters are murky, crocodiles lurk!"

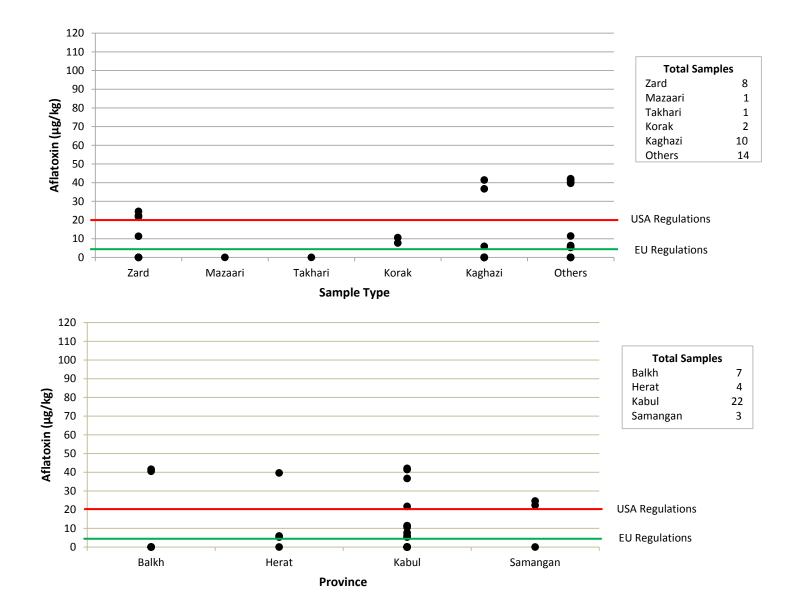
- Old African saying



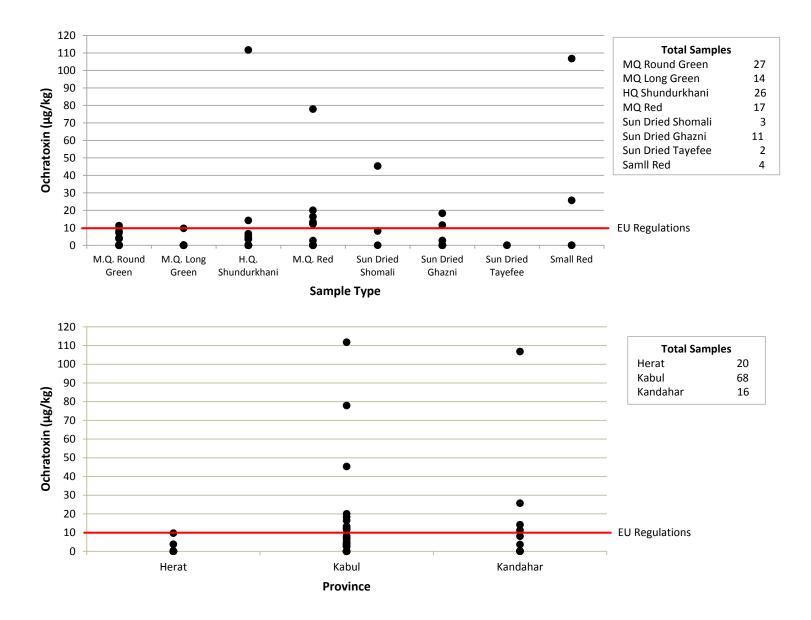
Almonds – Aflatoxins



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 Ghandi 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







COMMUNICATION IN THE CONTEXT OF FOOD SAFETY - RISKS AND OPPORTUNITIES -

Kabul, July 16, 2016

By Andja Cosic, CBCMP-II Communications Director



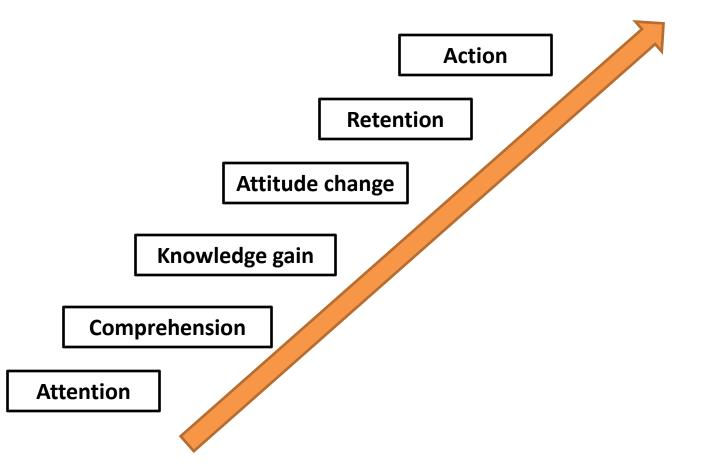








WHY DO WE COMMUNICATE ?













WHAT THE COMMUNICATION IS?













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











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	~	v	~	V	V	V	~	V
Average Literate	~	4	V	~	V	~	~	~
Nominally Literate	v	~	V					~
Illiterate	~							~

By Jeff Morris, Kansas State University



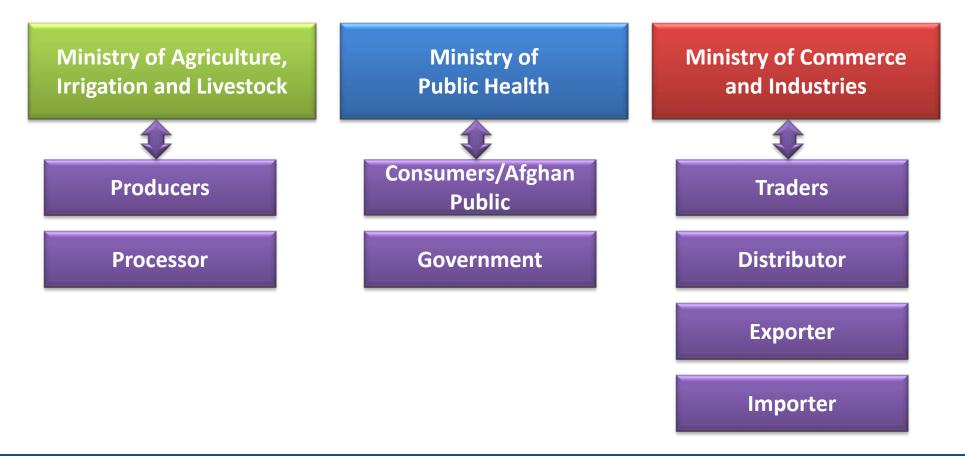








PRIMARY AUDIENCES BASED ON RESPONSIBILITIES













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.



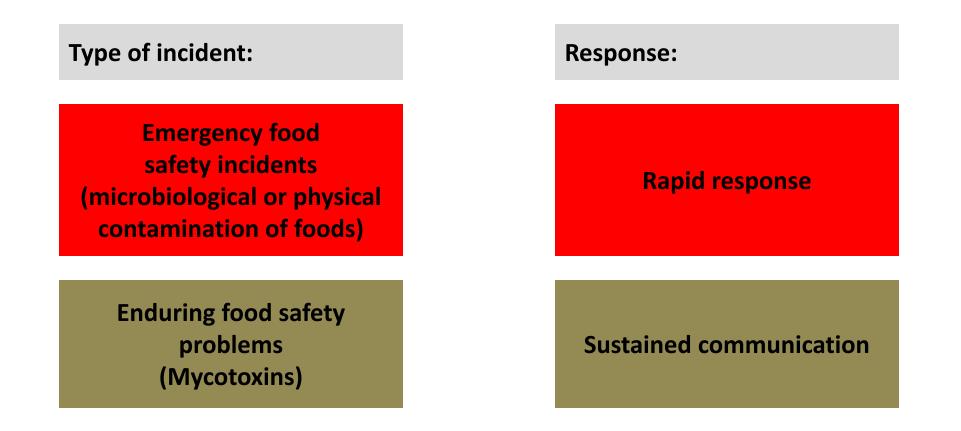








RISK COMMUNICATION TYPES IN FOOD SAFETY CONTEXT













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	Inclusion	Allow public	
and credibility	and dialog	inspection	
Messages adjusted to the audience	Good coordination	Adequate planning	











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.











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;











COMMUNICATING ON MYCOTOXINS IN AFGHANISTAN'S ENVIRONMENT - OPPORTUNITIES

Opportunity for the Government to show:

- \checkmark 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!

Strengthening human and institutional capacity of the Ministry of Agriculture, Irrigation and Livestock to better serve Afghan farmers and herders











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!

Strengthening human and institutional capacity of the Ministry of Agriculture, Irrigation and Livestock to better serve Afghan farmers and herders











Good communication is a learning opportunity and a trust building process! Andja Cosic

Thank you for your attention!







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

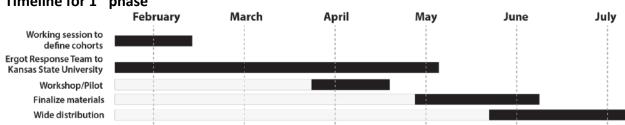
(i) The Afghan ergot response team will work directly with K-State counterparts to create highly specific visual communications materials and methodology for distribution. In addition, risk communications training will be conducted, including tracking and response mechanisms to determine outcomes of 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, MoCl 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





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? Whom we are talking to?/Who are our	Communicate within the MAIL about the problem. Identify the right person from ministries to follow up. Raise awareness of producers. Internal audience, to be informed and skilled to introduce new practices and			
audiences?	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.			



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Which communications	Direct communication with farmers	
tools and channels we are	through extension workers. Formal	
going to use?	trainings. Stakeholders meetings (i.e.	
	exporters and producers); Brochures;	
	Short educational videos;	
What needs to be done in	Committee to be established; training	
your Ministry to make it	needed; adequate planning;	
capable to communicate	communication; inter-ministerial	
about Mycotoxins?	committee; funding.	
Who from your Ministry	Most likely PPQD, however it requires	
could take the lead in	additional consultations prior final	
dealing with Mycotoxin	decision.	
issues, including		
communication?		
How we will monitor our	No answer at the moment;	
communication activities?		
How we will evaluate the	No answer at the moment;	
impact?		





Ministry of Commerce and Industries				
Questions	Answers	Level of Risk/Priority	Deadline	
What do we know about	- Impacts exports and reduces incomes;	Exports -	(When we can	
Mycotoxins? What are	- Diminishes country image;	medium,	start /how long	
the public health risks and	- Public health risks;	imports -	preparation is	
economic risks?	- Undermine counter narcotics efforts.	high	needed?)	
		priority.		
Whom to ask?/How to	- MAIL, MoPH, MoCl			
double-check?				
What are our	- Education and training of exporters;			
objectives?/What we	- HACCP;			
want to achieve	 Responsiveness (traceability); 			
communicating?	- Guarantee of safe food, consumer			
	protection;			
	- Compliance setting.			
Whom we are talking	Exporters; Processors; Producers; Value			
to?/Who are our	Chain actors; Importers; Distributors,			
audiences?	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	- Over or underestimate audience			
and risks we have to take	capacity.			
into account?	- Correct understanding of business			
	practices;			
	- Decrease in trade volumes.			
What needs to be done in	- EPAA needs to be strengthened;			
your Ministry to make it	- Strengthen technical unit at MoCI that			
capable to communicate	has laboratory facilities and often clears			
about Mycotoxins?	products for export (also referred as			
	Association of exports of dried fruits);			
	- Identify international best practices;			
Who from your Ministry	To be identified (technical unit alluded to			
could take the lead in	above).			
dealing with Mycotoxin				
issues, including				
communication?				
Which communications	Printed instructions and info brochures			
tools and channels we are	about standards, regulations, best			
going to use?	practices etc.; Trade groups; Civil society;			



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	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?	 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?	 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?	 Minimize the risk. Improve health and nutrition status of the people. 			
Whom we are talking to?/Who are our audiences?	 Health providers/medical workers. General population. Decision makers. 			
What are our messages?	 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?	 - 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: - mass media, - brochures, - trainings, - public gatherings, mosques.			
What needs to be done in your Ministry to make it capable to communicate about Mycotoxins?	 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	 Food safety, Nutrition, and Health Promotion Departments. 			



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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. 	