

Afghanistan

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Five Agriculturally Most Important (Regulated) Mycotoxins

- Aflatoxins
- Fumonisin
- Deoxynivalenol and other trichothecenes,
e.g., T-2
- Zearalenone
- Ochratoxin

What Was Done?

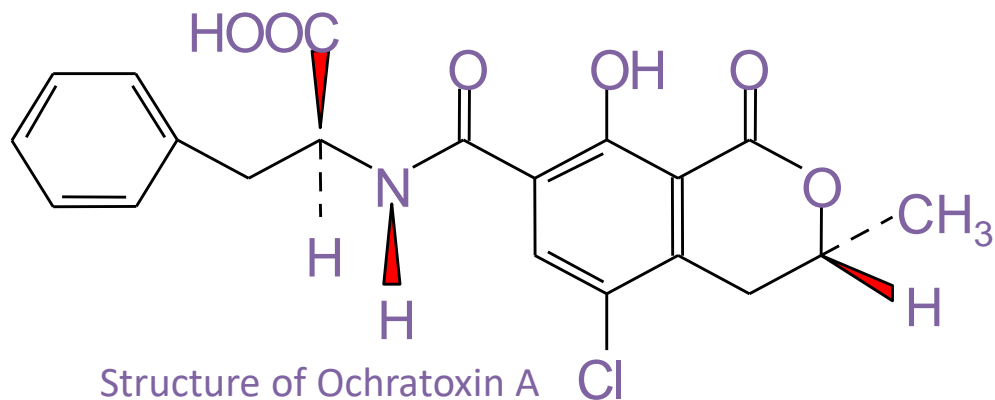
- Afghanistan – MAIL and Deb Frey
 - Trained staff
 - Established and equipped a functional lab
 - Collected samples from across Afghanistan
 - Assayed raisins, nuts & wheat with Romer test kits
- Italy – ISPA
 - UPLC and LC-MS assays for trichothecenes in wheat
 - Mycological analyses of flour
- Austria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



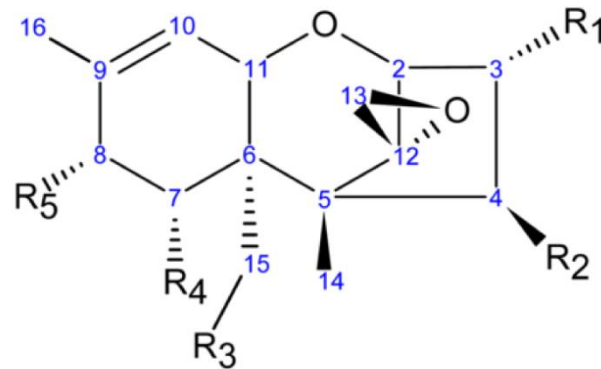
Kidney failure
Cacao
Nuts
Grapes
Coffee
Wheat



Trichothecenes

- Two classes – A & B, strains make only one type
- Both inhibit protein synthesis
- Most common in grains
- Type A – very toxic – T-2, HT-2 & DAS
 - US Select agent list
 - Purportedly used for biological warfare
- Type B – not as toxic – DON, NIV
 - More widespread, especially on wheat
- Can be taken up through skin or intestinal mucosa
- Cause vomiting, diarrhea, & immune suppression

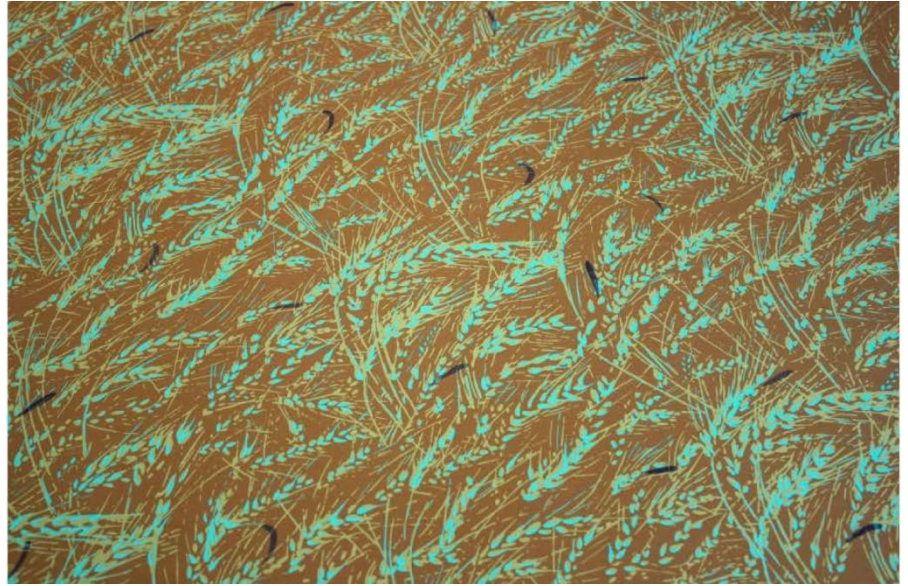
Toxin	R1	R2	R3	R4	R5
DON	-OH	-H	-OH	-OH	=O
3-ADON	-OAc	-H	-OH	-OH	=O
15-ADON	-OH	-H	-OAc	-OH	=O
NIV	-OH	-OH	-OH	-OH	=O
T-2	-OH	-OAc	-OAc	-H	-Olsoval
HT-2	-OH	-OH	-OAc	-H	-Olsoval
4,15-DAS	-OH	-OAc	-OAc	-H	-H



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

Ergot Alkaloids

- Small Grains – Wheat, rye, barley & oats
- An unexpected finding by Austrian group
- Not highly regulated (animal feed only)
- In small doses – hallucinations (LSD)
- In other cases – neuropathy and gangrene
- Gnostics and ancient Greeks may have used them to help people have visions
- Controlled by sorting ergot bodies from the grain before processing



Some Ergot Epidemics

-600 – Assyria

857 – Germany

945 – France

1093 – France

1692 – USA

1926 – Russia

1929 – Ireland

1953 – France

1958 – India

1973 – Ethiopia

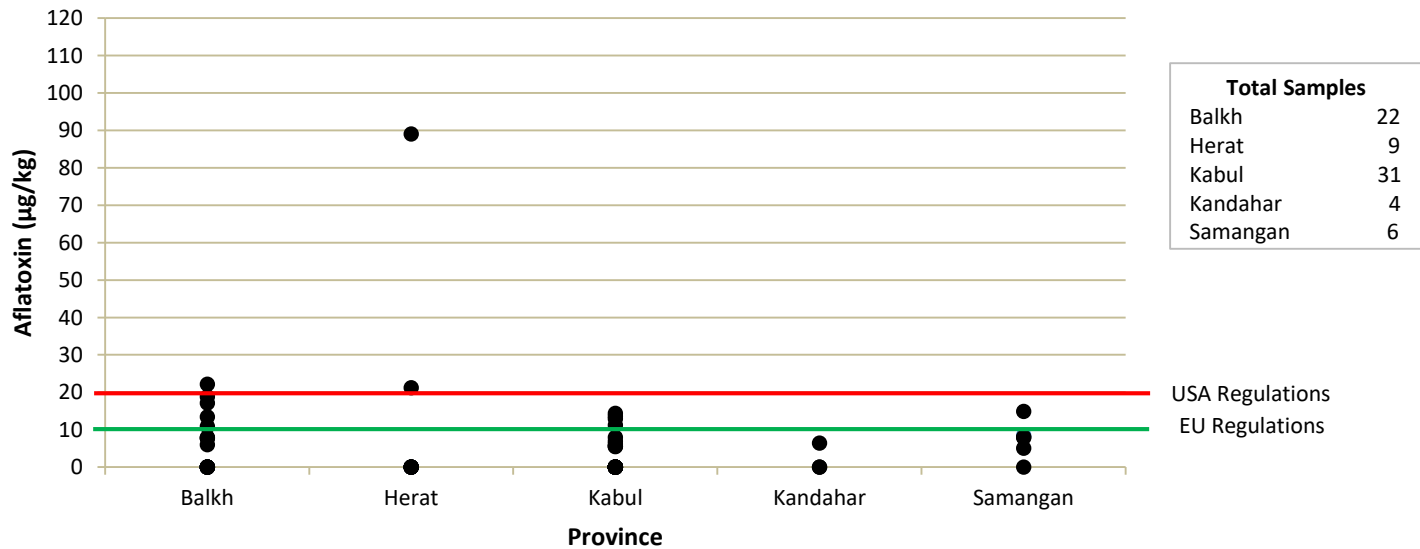
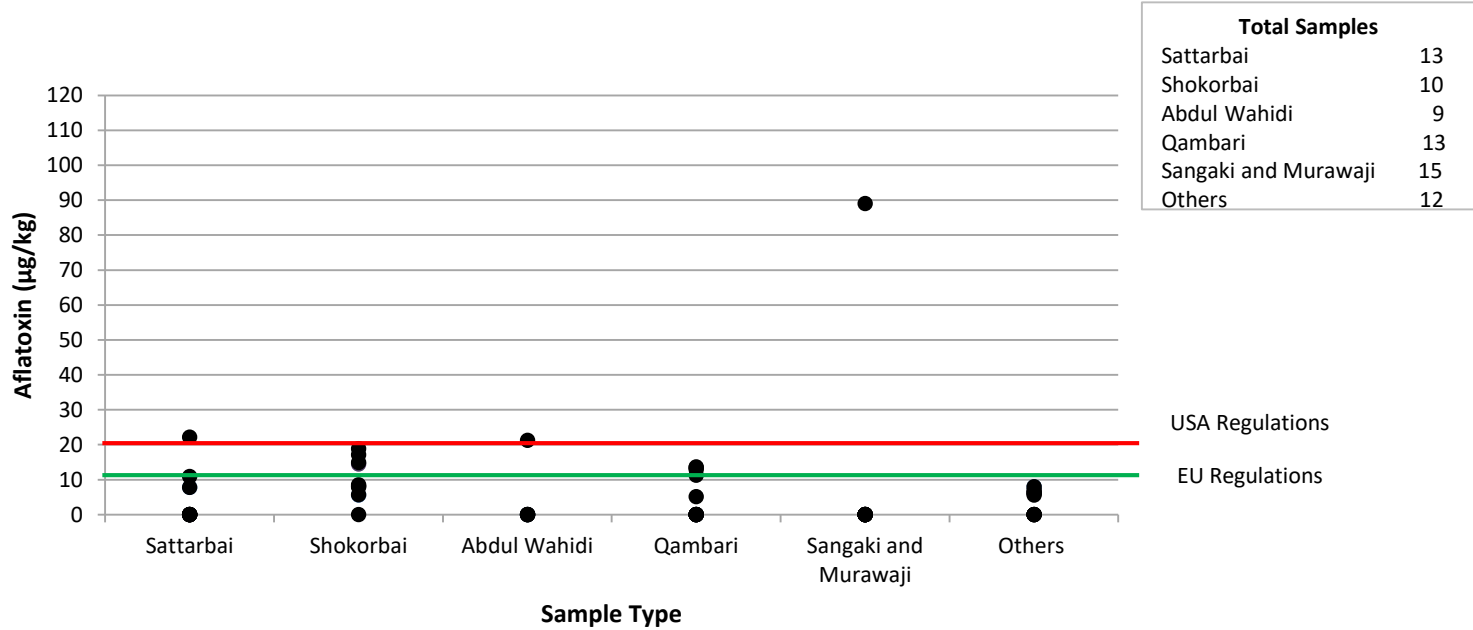
Test Kit Performance

- Romer kits for aflatoxin, ochratoxin and deoxynivalenol (DON or vomitoxin) were reliable for all tested substrates
- Vicam kits for ochratoxin and DON were reliable for wheat
- Neogen tests for aflatoxin and ochratoxin were reliable for all tested substrates
- Vicam kit for aflatoxin did not work with wheat
- Romer test for T-2 toxin was erratic
- Neogen and Romer tests for T-2/HT-2 toxins gave many (Romer) and exclusively (Neogen) false positives in wheat

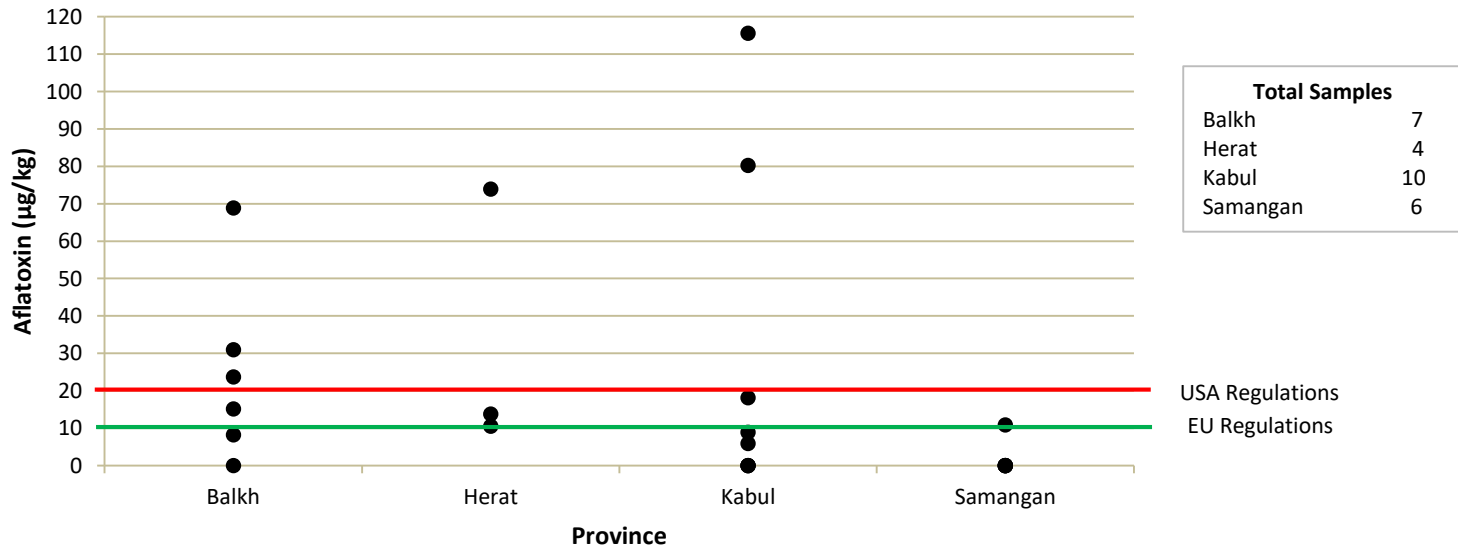
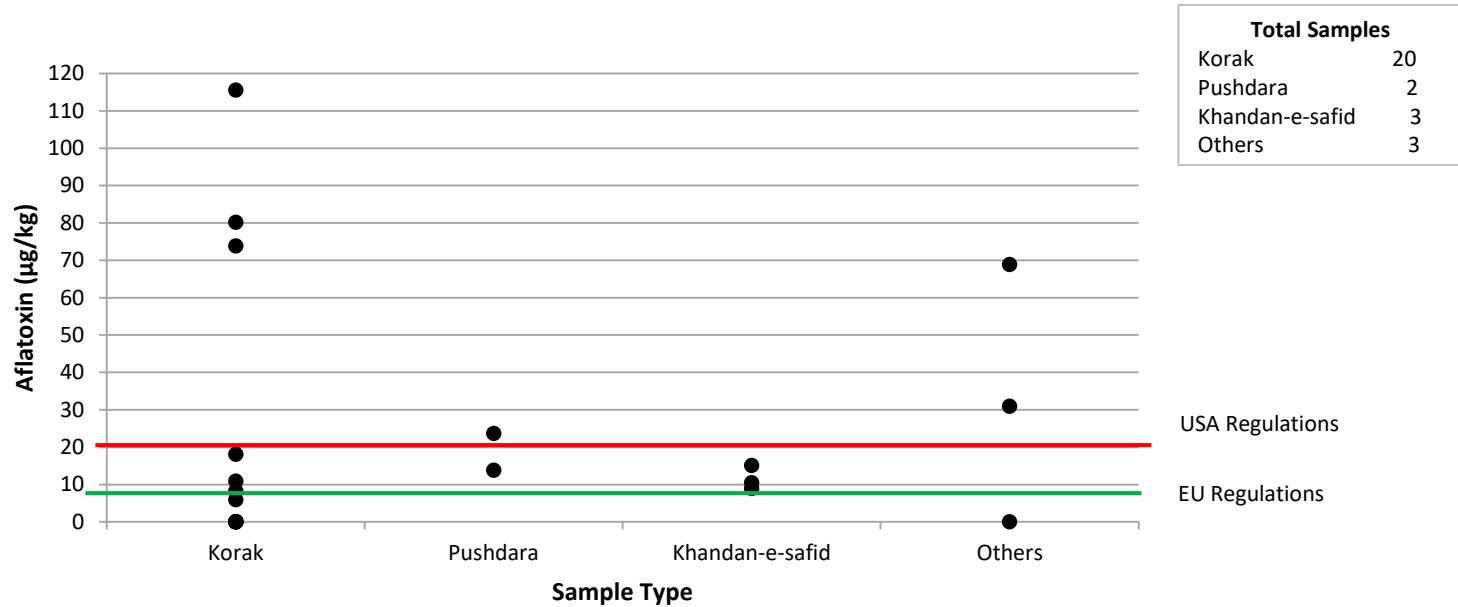
Nuts – Results

- Aflatoxin
 - Almonds – 15/81 at export limiting level
 - Pistachios – 19/40 at export limiting level
 - Walnuts – 8/25 at export limiting level
- Ochratoxin
 - Almonds – no contamination detected
 - Pistachios – 2/40 at export limiting levels
 - Walnuts – no contamination detected

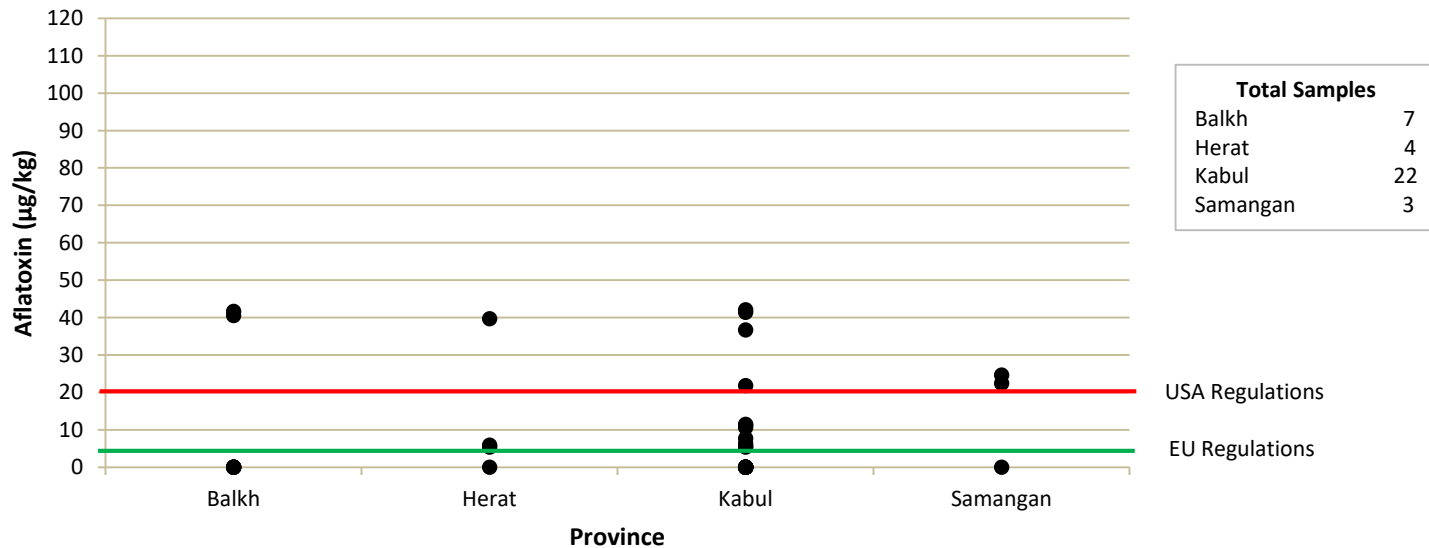
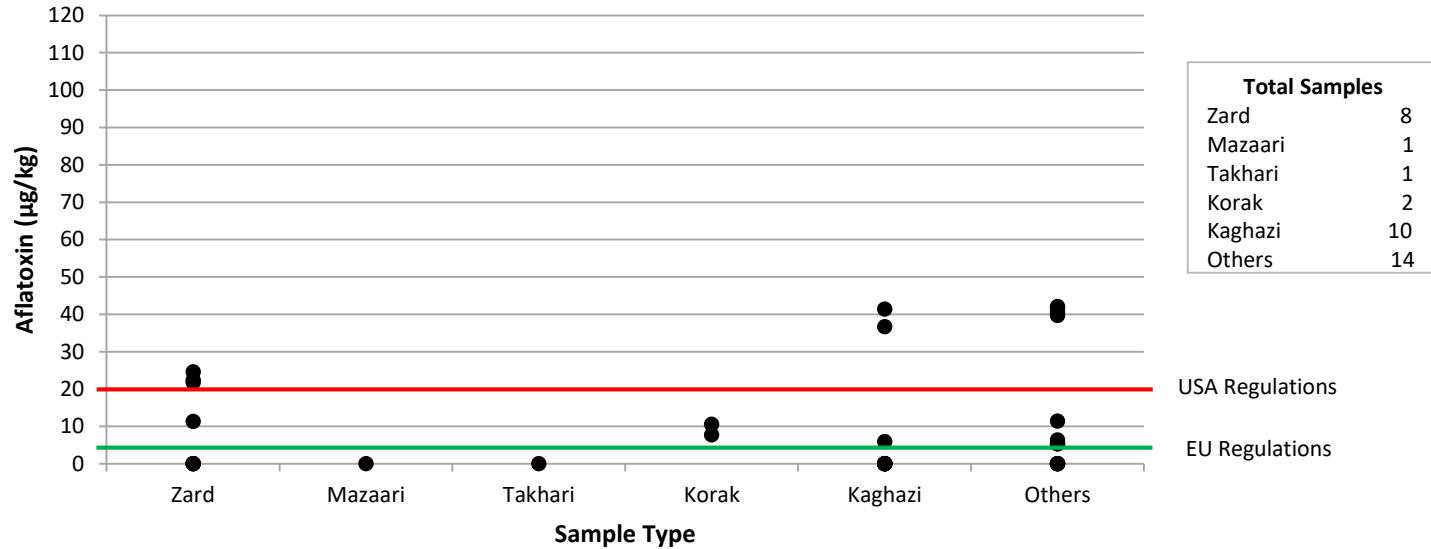
Almonds – Aflatoxins



Pistachios – Aflatoxins



Walnuts – Aflatoxins



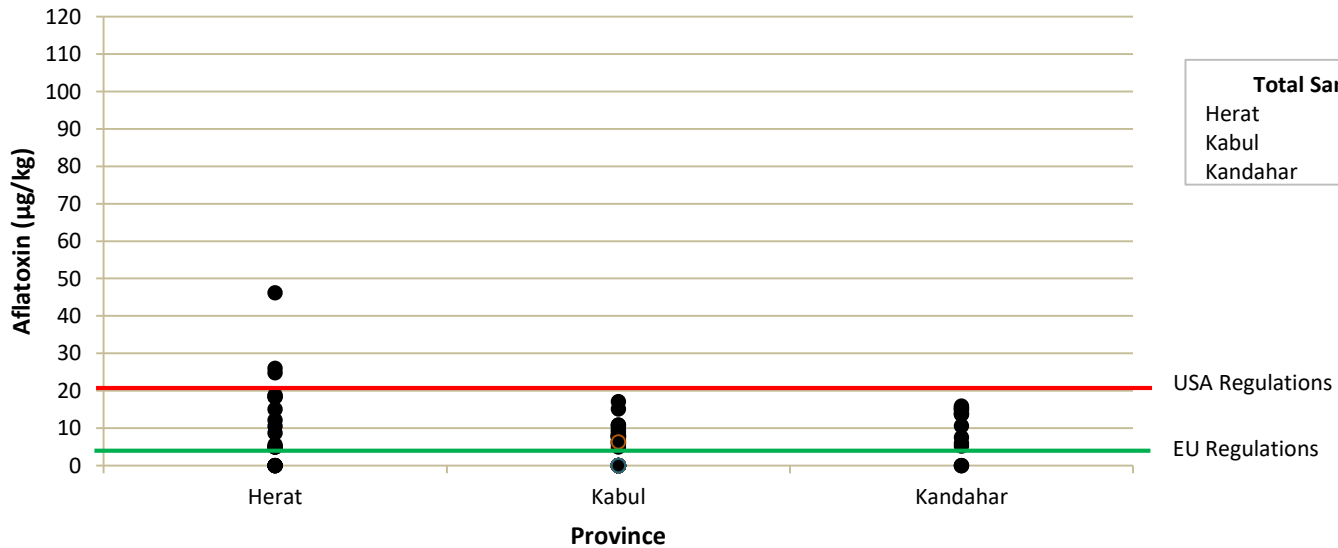
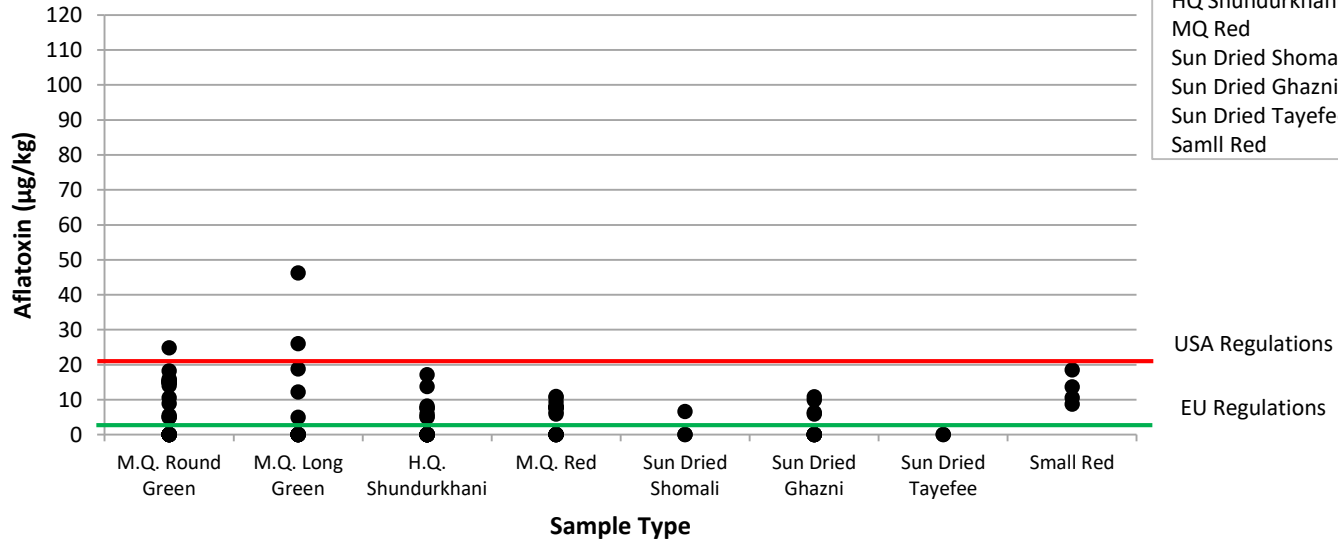
Austrian Screen – Nuts

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

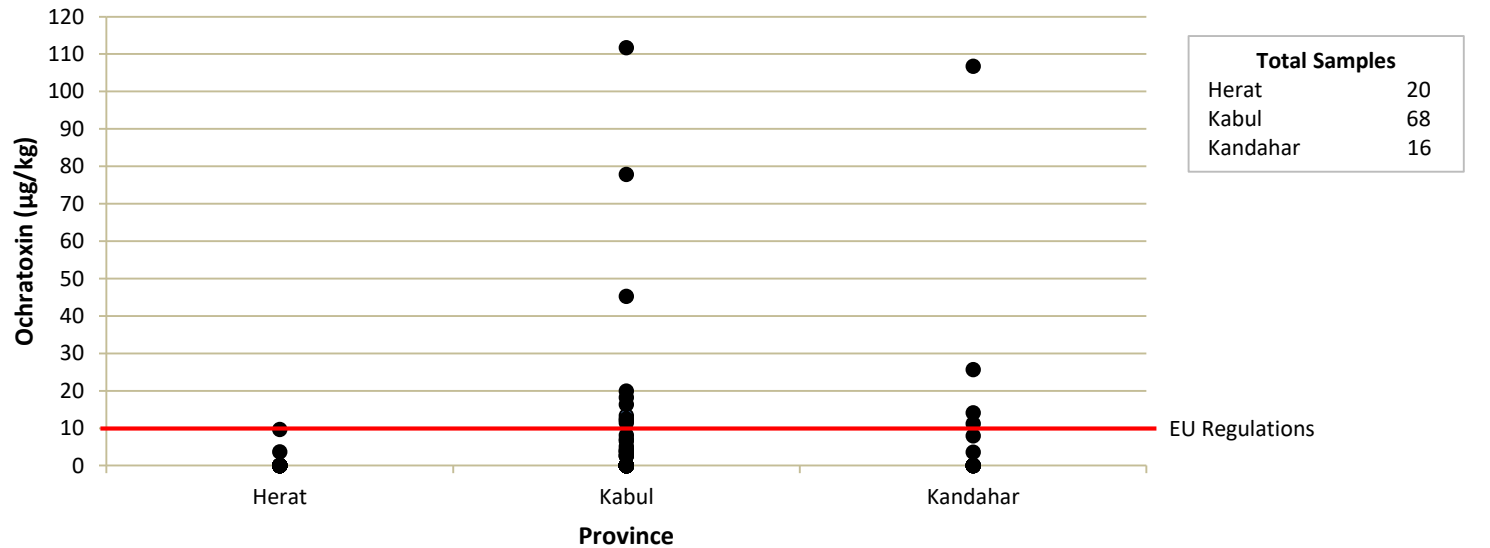
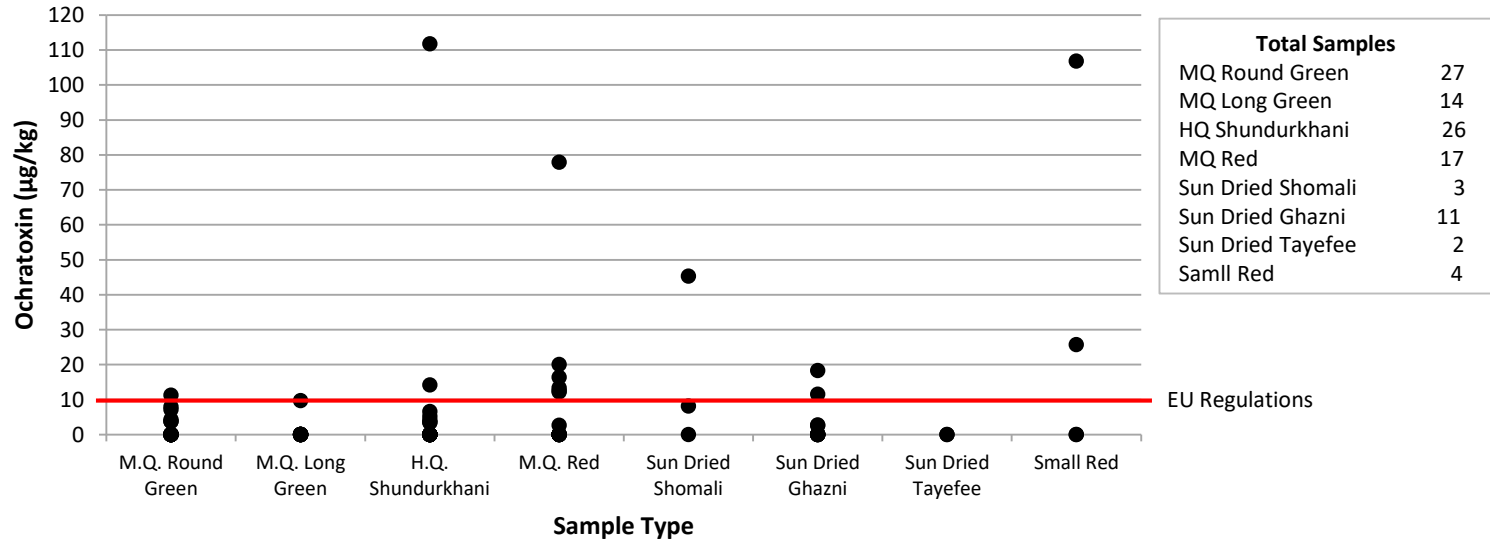
Raisins – Results

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

Raisins – Aflatoxins



Raisins – Ochratoxin



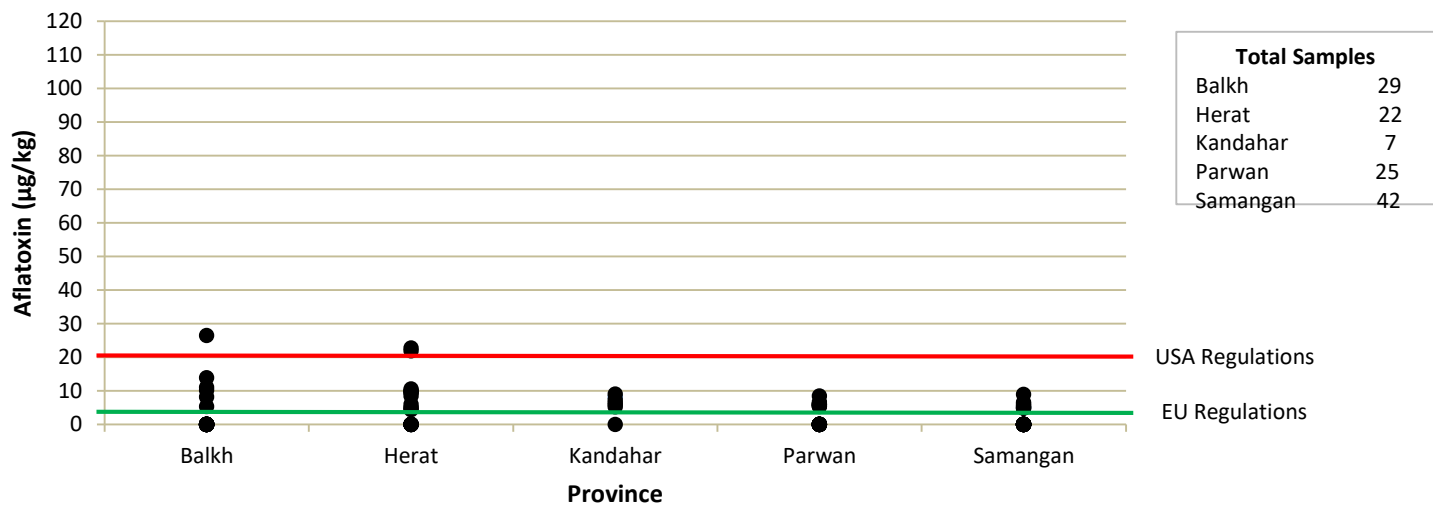
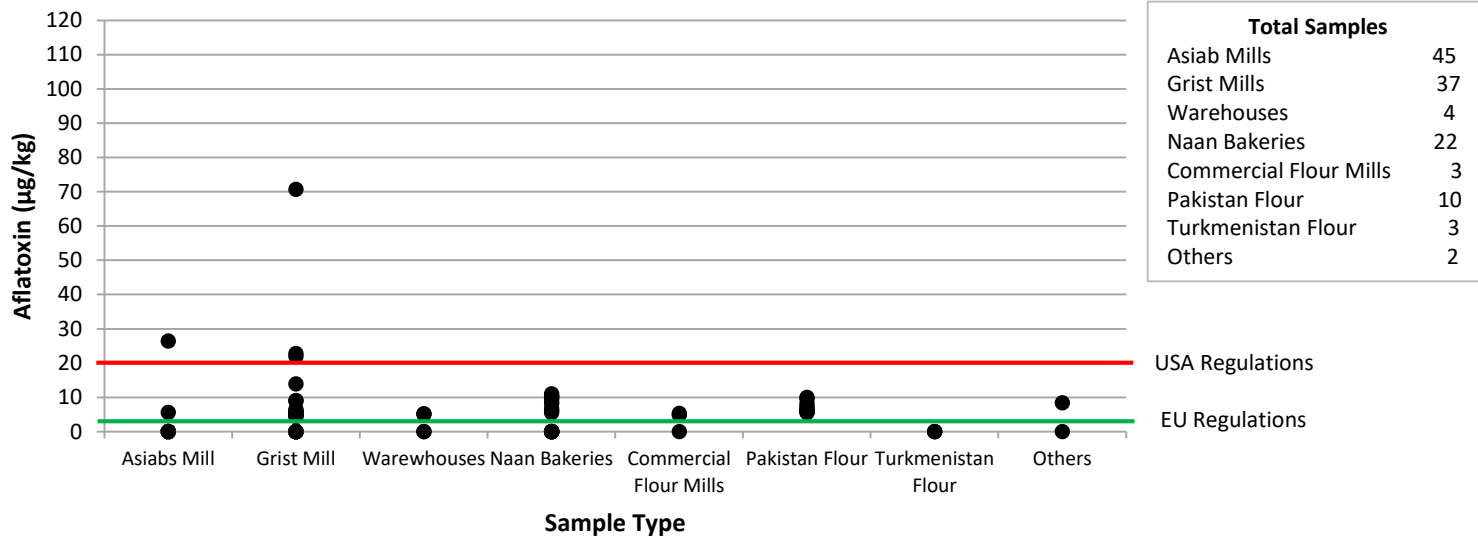
Austrian Screen – Raisins

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

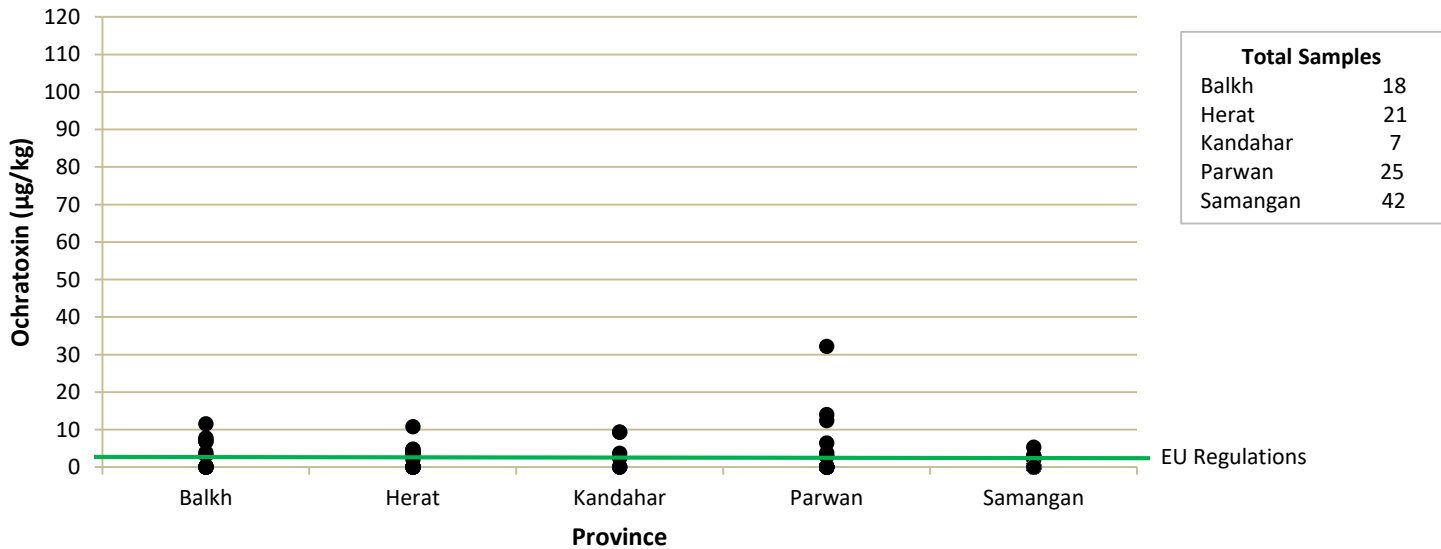
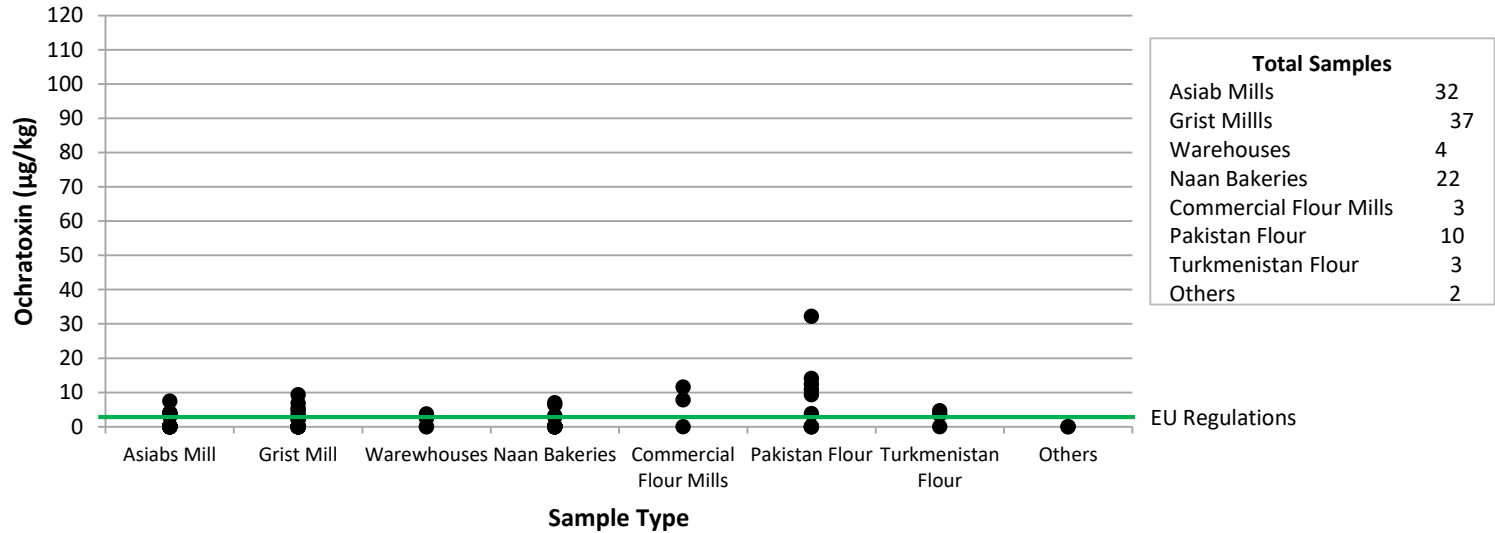
Wheat – Results

- International standards may be too high for Afghanistan safety because of the large amount of wheat consumed daily (500 g/person/day)
- Aflatoxins – detectable in 23/151 samples
 - Not a field contaminant of wheat
 - Contamination likely occurs in storage
- DON – 3/185 above international guidelines
 - Not a major problem, but exists
 - Weather and storage dependent
- Ochratoxin – detectable in 36/181
 - Common problem in northern Europe
 - Needs attention
 - May carry over to meat
- T-2 and HT-2 – Not reliably detected
- Ergot – detectable 51/151
 - High incidence
 - Easily remedied by cleaning grain

Wheat - Aflatoxin



Wheat – Ochratoxin



Austrian Screen – Wheat

<i>Fusarium</i>	<i>Alternaria</i>	<i>Aspergillus</i>	<i>Penicillium</i>	<i>Claviceps</i>
Beauvericin	Alternariol	Aflatoxin	Agroclavine	Ergocristine
Enniatin A	Alternariol methyl ether	Averantin	Chanoclavine	Ergocristinine
Enniatin A ₁	Altersetin	Averufin	Citrinin	Ergometrine
Enniatin B	Altersolanol	Cycloaspeptide A	Chrysogine	Ergometrinine
Enniatin B ₁	Altერთoxin-I	Kojic acid	Elymoclavine	Ergosin
Epiequisetin	Macrosporin	Methoxysterigm atocystin	Mycophenolic acid	Ergosinin
Equisetin	Tentoxin	3-Nitropropionic acid	Questiomycin A	Ergotamine
HT-2 toxin	Tenuazonic acid	Norsolorinic acid	Quinolactacin A	Ergotaminine
T-2 toxin		Ochratoxin	Secalonic acid D	
Zearalenone		Sterigmatocystin		

Conclusions

- The test kit used matters
- Sampling procedures are critical
- Pre- and Post-harvest processes both matter
- Nuts and aflatoxins – Pistachios > Walnuts > Almonds
- Raisins – Aflatoxins > Ochratoxin
 - Type of raisin and drying method important
- Wheat
 - Need to evaluate safety levels for Afghan diets
 - Aflatoxin is a storage issue
 - *Fusarium* toxins (T-2, HT-2, DON & Zearalenone) are minimal
 - Citrinin + ochratoxin could enhance kidney problems
 - Ergot – high frequency, but relatively easy to fix

Questions?

“Where waters are
murky, crocodiles lurk!”

- Old African saying

