

Aflatoxins – What, Where and Why Test



Stephen Holmes
Charm Sciences Inc.
November 7, 2013

Introduction

- Mycotoxins - where does it come from?
- What is Aflatoxin?
- Why is Aflatoxin of concern?
- How to monitor or test for Aflatoxin?

Mycotoxins – where does it come from?

(Myco) or **Mykes**: Greek for fungus/mold

(Toxin) or **Tocicum**: Latin for poison/toxin

Mycotoxins – where does it come from?

Mycotoxins produced by 3 main genera of Fungi

- Aspergillus
- Penicillium
- Fusarium



Mycotoxins – 6 major types

Aflatoxin: Carcinogenic (affects liver)

DON (Vomitoxin): Feed refusal, immunosuppressive activity

Fumonisin: Carcinogenic, equine leukoencephalomalacia, and pulmonary edema in swine

Ochratoxin: Porcine & Poultry nephropathy, reduced feed intake

T2/HT2: Feed refusal, immunosuppressive activity

Zearalenone: Estrogenic toxin, swine and poultry susceptible

What is Aflatoxin?

- Aflatoxin was first identified in early 1960's
- Aflatoxin is a natural toxin, and potent carcinogen, produced by the fungus *Aspergillus flavis* and *Aspergillus parasiticus*
- There are 4 Aflatoxins produced by fungus growth in grains: B1, B2, G1, G2. B1 is the predominate form
- Aflatoxin B1 when ingested by animals is converted to Aflatoxin M1. This is the predominate Aflatoxin found in milk
- Aflatoxins can occur pre-harvest, in the field, or post-harvest, due to delayed drying and/or improper storage at moisture levels



What is Aflatoxin?

- Conditions that favor aflatoxin production:
 - High moisture ($\geq 14\%$)
 - Drought stress
 - Insect damage
- Highly regulated worldwide

What is Aflatoxin?

Mold: Pre-Harvest



What is Aflatoxin?

Mold: During Storage

Properly stored corn



Corn exposed to moisture for 18 days



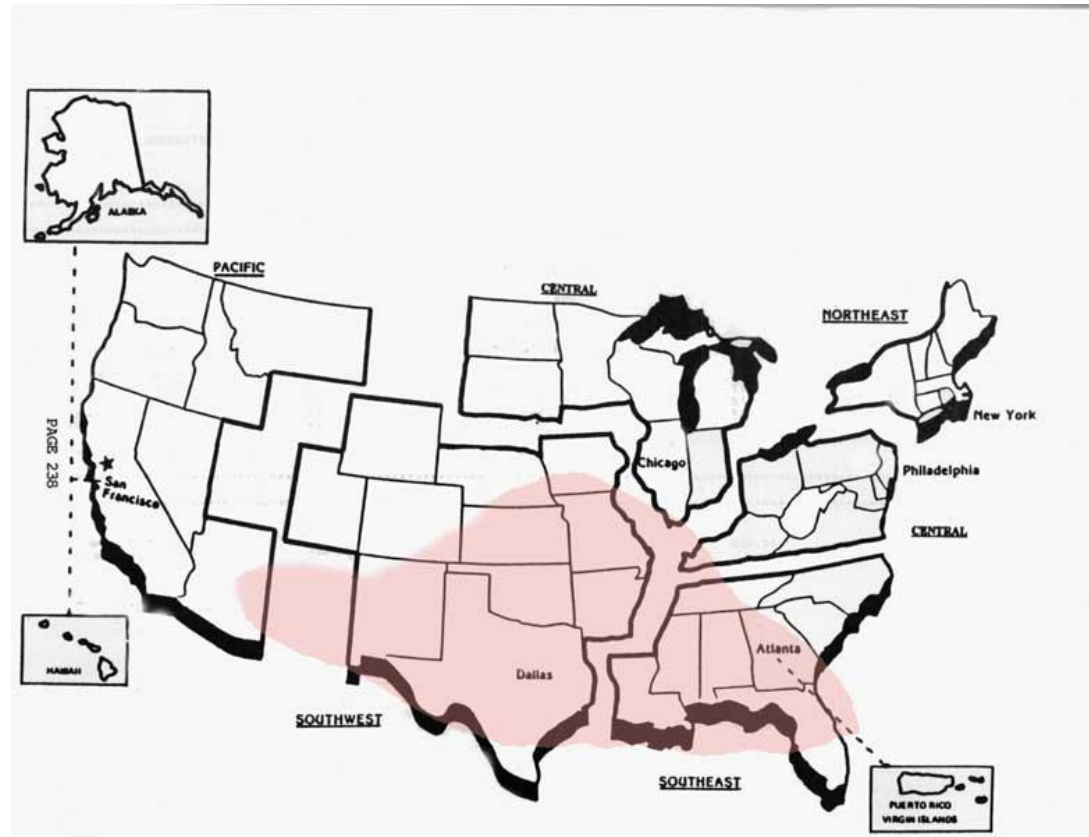
Aflatoxin in History

Fact: Lord Carnarvon and 26 of his colleagues and associates met with “mysterious” deaths following the opening of King Tut’s tomb. Fiction: Deaths were the result of King Tut’s curse. Scientific studies revealed the presence of *Aspergillus* mold spores (extremely poisonous) in the tomb were at a high level when the tomb was opened. Inhalation or ingestion of the spores are the cited source for the numerous and mysterious deaths — the toxic mold had lain dormant in King Tut’s tomb for thousands of years before stones were removed for Lord Carnarvon’s and others’ entry. The wisps of in-rushing air and movement of all entering disturbed and dispersed the toxic spores into the air.

(In a similar story dating in the early 1970’s, 10 of the 12 scientists not wearing respirators and who unsuspectingly entered a mold infested Polish royal tomb, succumbed to the mold’s toxic effects.)

Climates Favorable to Aflatoxin in US

- Hot and Dry
- Semi-arid
- Humid

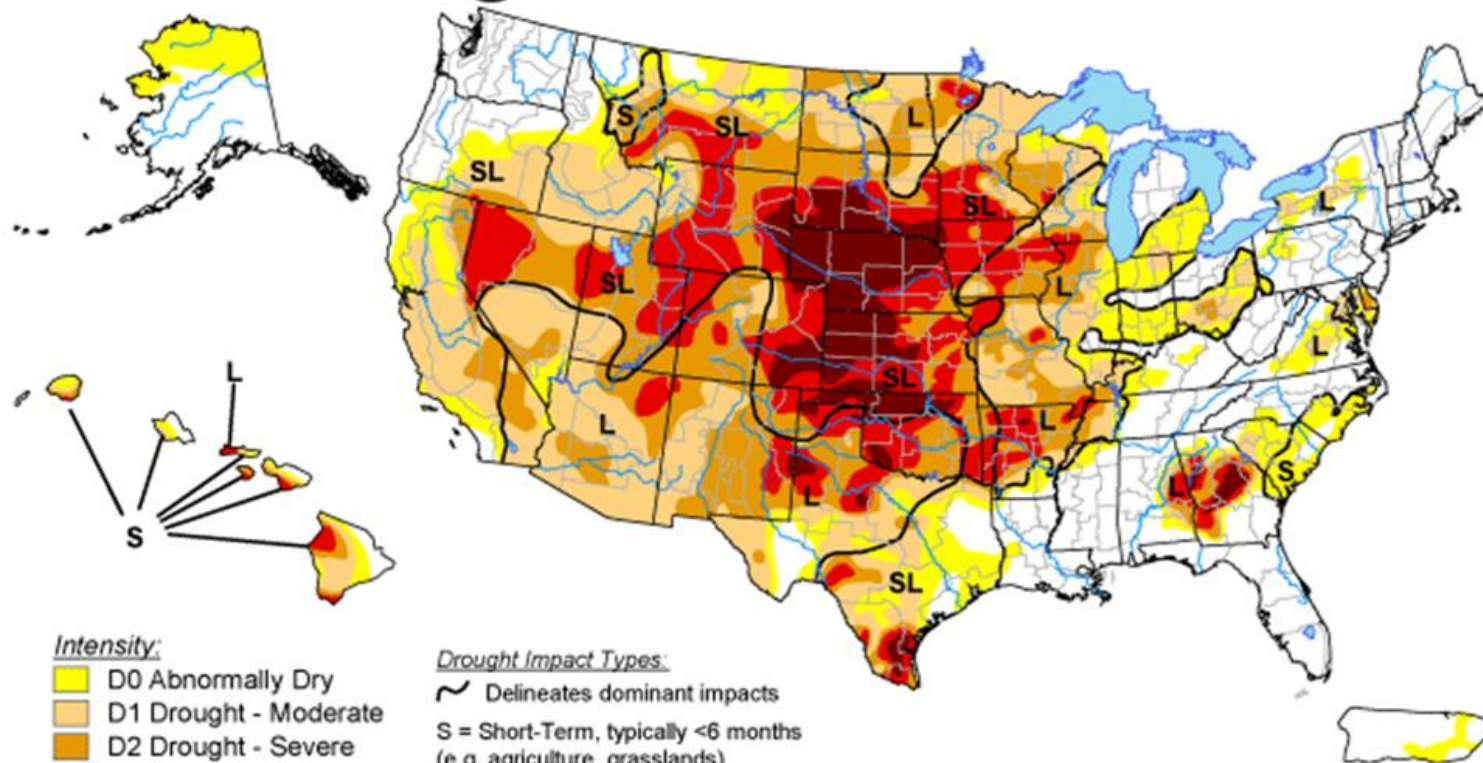


Improperly dried and stored
moist corn

U.S. Drought Monitor

October 23, 2012

Valid 7 a.m. EDT



Intensity:

- D0 Abnormally Dry
- D1 Drought - Moderate
- D2 Drought - Severe
- D3 Drought - Extreme
- D4 Drought - Exceptional

Drought Impact Types:

- Delineates dominant impacts
- S = Short-Term, typically <6 months
(e.g. agriculture, grasslands)
- L = Long-Term, typically >6 months
(e.g. hydrology, ecology)

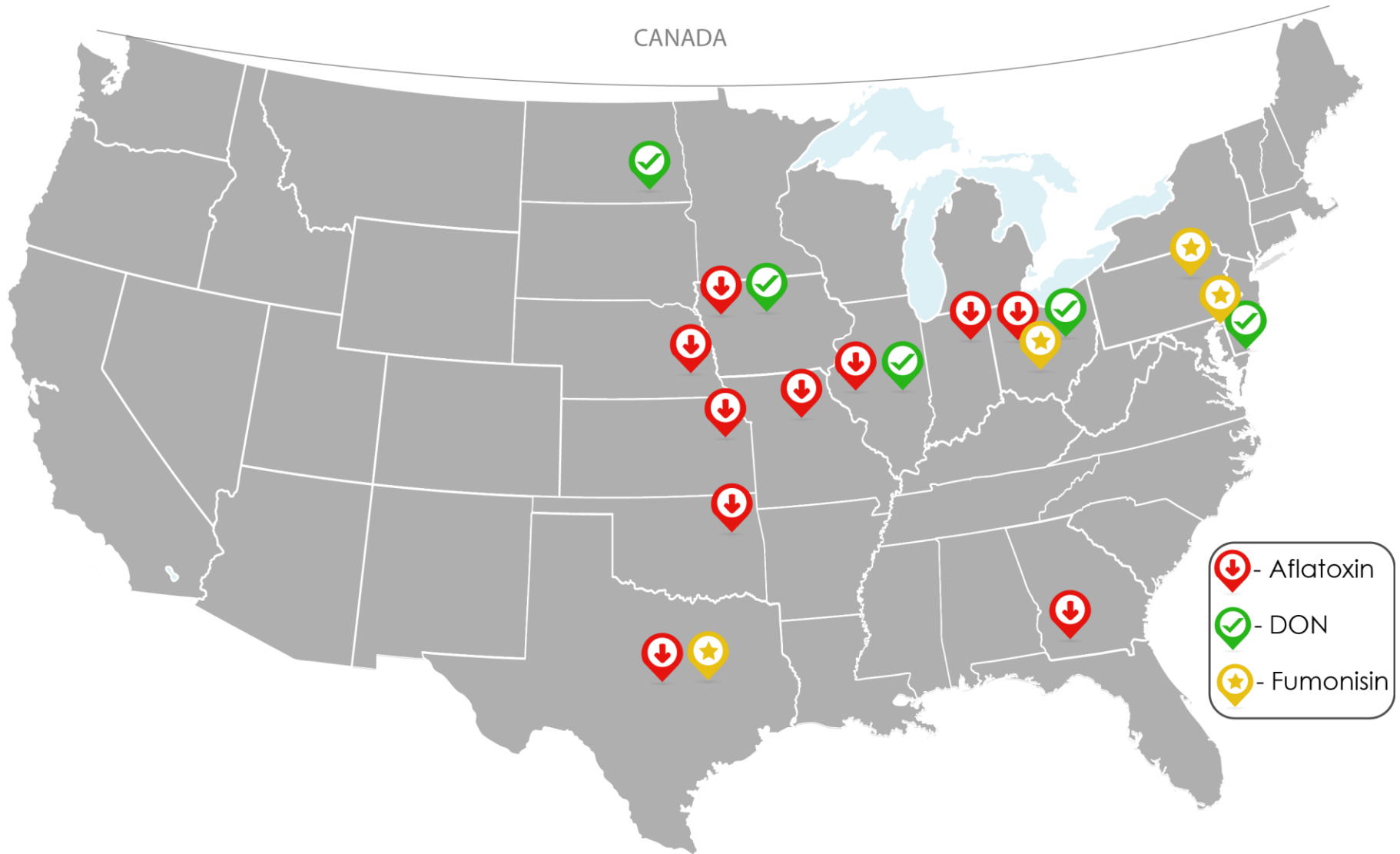
The Drought Monitor focuses on broad-scale conditions.
Local conditions may vary. See accompanying text summary
for forecast statements.

<http://droughtmonitor.unl.edu/>

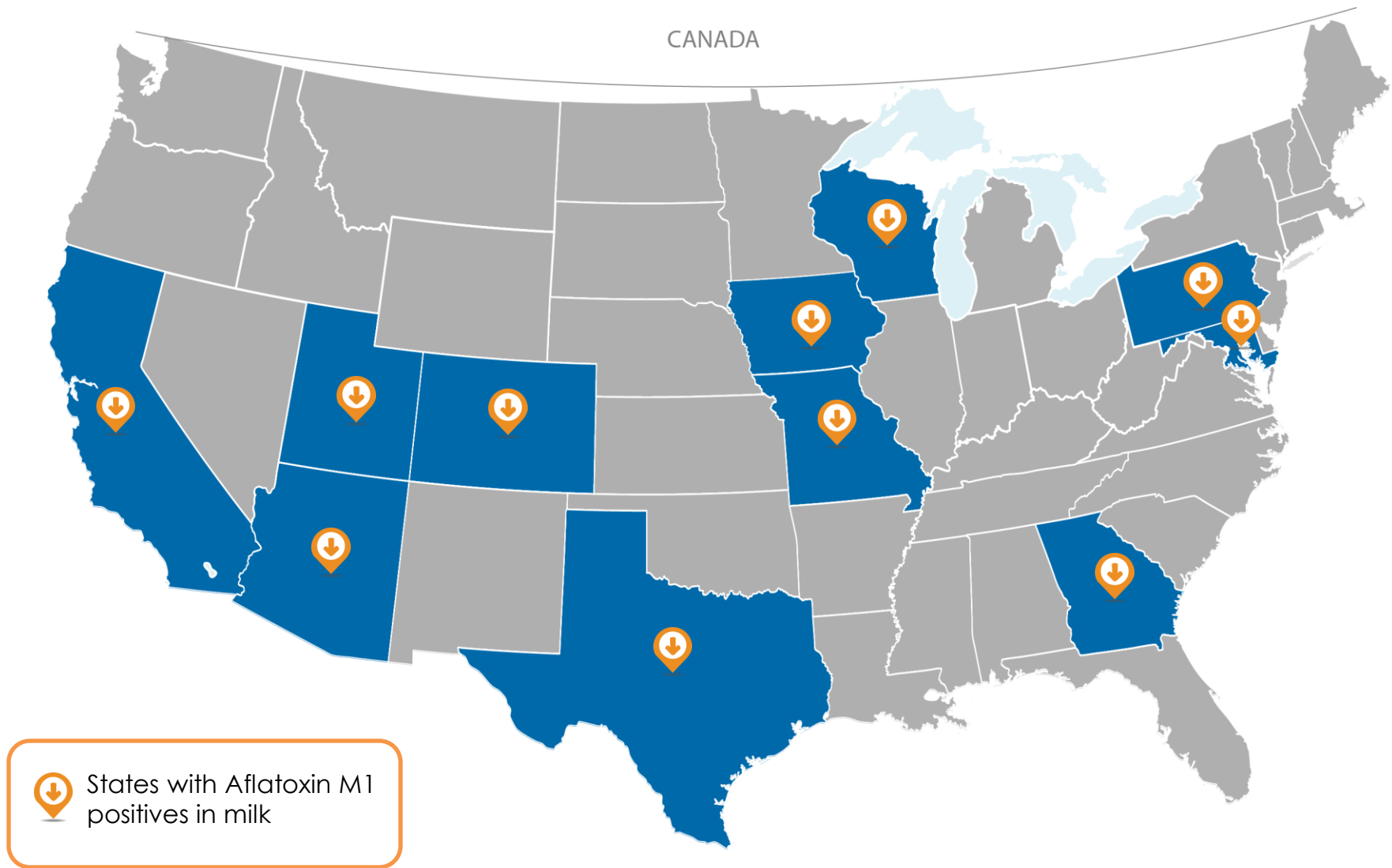


Released Thursday, October 25, 2012

Author: Brad Rippey, U.S. Department of Agriculture



Mycotoxins Reported in 2012



Aflatoxin M1

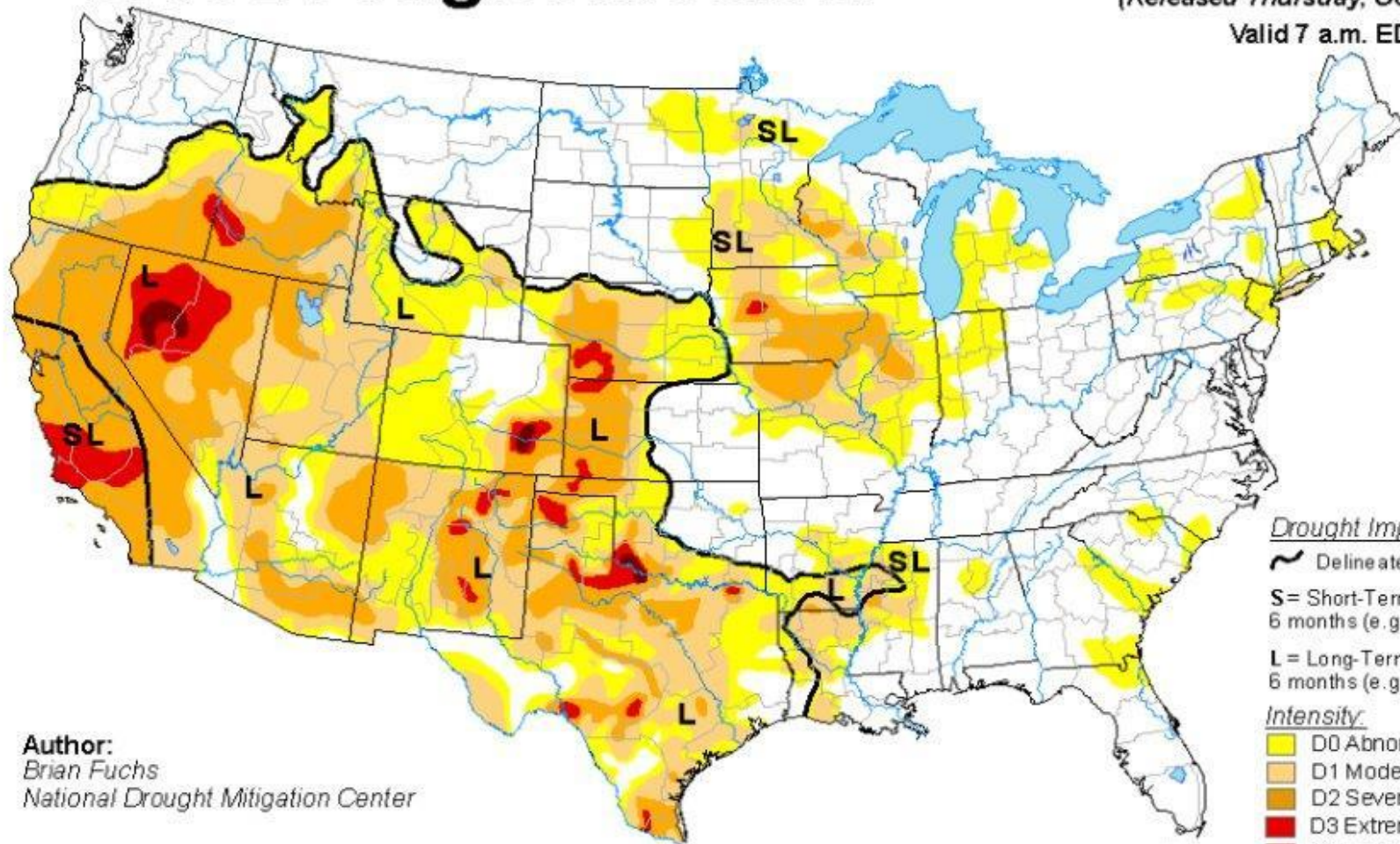
Positives in Milk-Map 2012

U.S. Drought Monitor

October 22, 2013

(Released Thursday, Oct. 24, 2013)

Valid 7 a.m. EDT



Author:
Brian Fuchs
National Drought Mitigation Center

Drought Impact Types:

~ Delineates dominant impacts

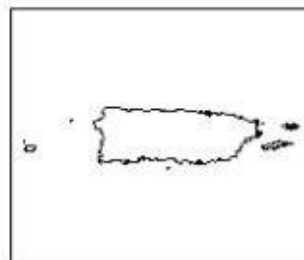
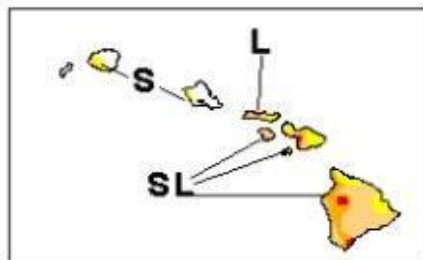
S = Short-Term, typically less than 6 months (e.g. agriculture, grasslands)

L = Long-Term, typically greater than 6 months (e.g. hydrology, ecology)

Intensity:

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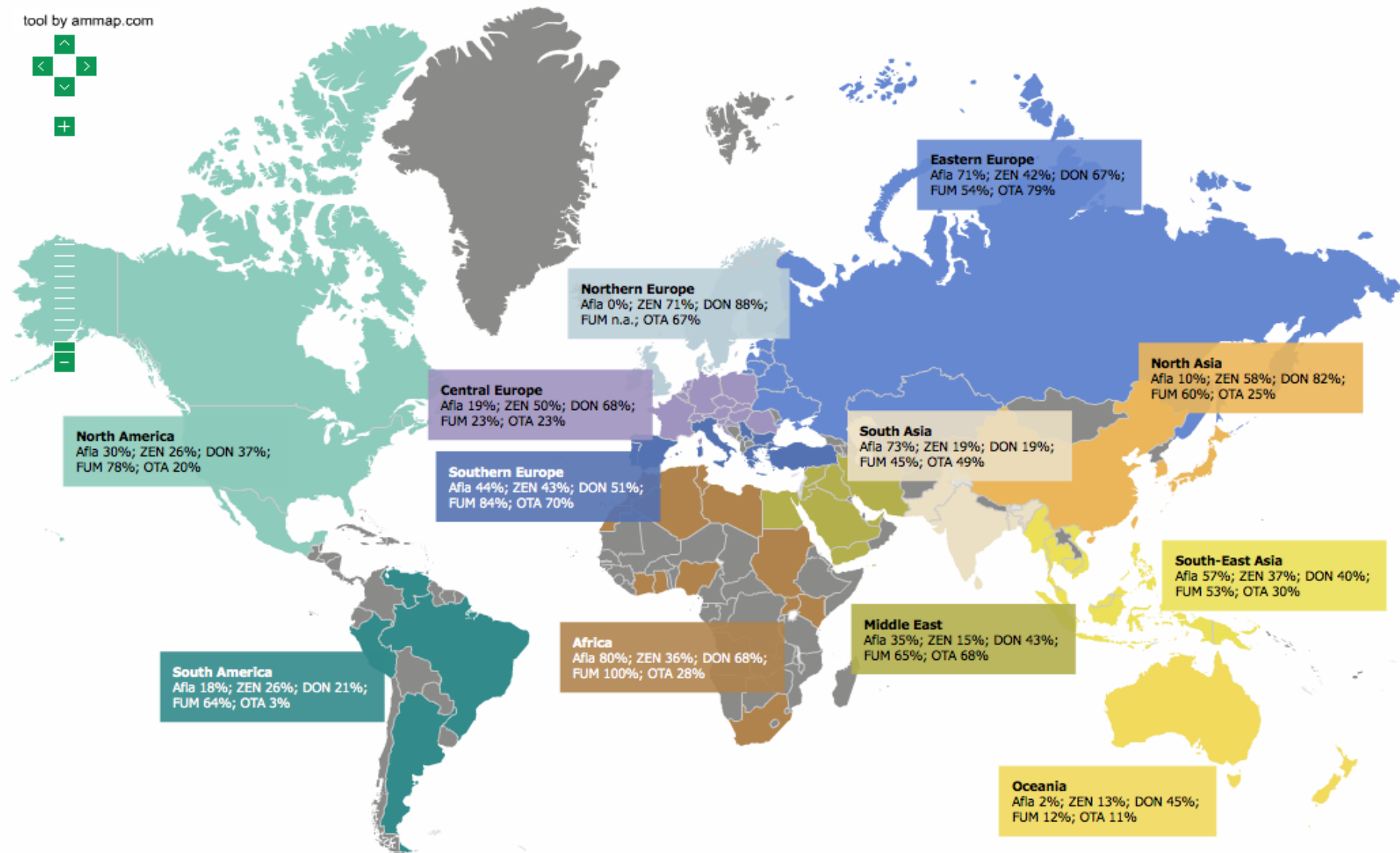
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<http://droughtmonitor.unl.edu/>

Prevalence of Mycotoxins

tool by ammap.com



Incidence of Aflatoxin contamination in Europe – 2012 & 2013

EU: Feed contaminated with Aflatoxin



PROCESS MANAGEMENT AFLATOXIN FEED CONTAMINATION MAIZE PROFIT STATISTICS MILLS
DIOXIN RE-CALLS CATTLE GERMANY NETHERLANDS US

2016 X

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Authorities in the Netherlands and Germany are scrambling to detect and remove from circulation tonnes of feed potentially contaminated with aflatoxin, according to recent press report . The source is presumed to be a consignment of 45,000 million tonnes of corn imported from Serbia and distributed by Toepfer.



Approximately 10,000 tonnes of the suspect batch was distributed to 13 feed mills in Northern Germany resulting in delivery of feed to 4,000 farms in Lower Saxony and numerous small operations in seven other states. Product was also shipped to the Netherlands where trace-forward is in progress.

The affected consignment allegedly assays at 200 ppb aflatoxin, approximately 10 times the permitted level. This corn would raise the level of aflatoxin-M in milk above the US statutory limit of 0.5 ppb at even low levels of dietary inclusion. Now concern over contamination is leading to widespread recall of dairy products in the affected regions, reminiscent of previous dioxin and PCB contamination episodes.

Is Global Warming influencing Mycotoxin Growth?

- Northern climates being affected by:
 - Warmer temperatures
 - Bigger weather events
- Larger dispersion/transportation of grain world wide

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GREATER RISK FOR MYCOTOXIN PROBLEMS

Economic Impact of Mycotoxins - 2012

More than 80 percent of the acres of major field crops planted in the United States are covered by Federal crop insurance, which can help to mitigate yield or revenue losses for covered farms. As of September 30, only 25 percent of the crop was rated good to excellent with 50 percent rated poor or very poor.

<http://www.ers.usda.gov/newsroom/us-drought-2012-farm-and-food-impacts.aspx>

Agricultural economists at the University of Illinois estimate the drought will trigger this year gross indemnities of roughly \$30bn, with an underwriting loss of \$18bn. Of that, the US government would shoulder around \$14bn, while private sector insurers are likely to face a loss of \$4bn, they said. Standard & Poor's, the rating agency, put the losses of the private sector a notch higher at \$5bn.


"The US drought is indeed a 'catastrophic' event," Gregory W Locraft, insurance analyst at Morgan Stanley in New York, wrote in a recent note to clients, adding that it "is likely the largest [insurance] crop loss in history."


<http://news.firedoglake.com/2012/08/27/hidden-costs-drought-crushing-crop-insurance-industry/>

Mycotoxin Status Update 2012

- Six states – Illinois, Indiana, Iowa, Kansas, Nebraska and Oklahoma – applied for and received U.S. Food & Drug Administration waivers to allow corn containing more than 20 parts per billion (ppb) of aflatoxin to be blended with corn containing lower levels of aflatoxin for use as animal feed. Similar waivers were granted in 2005, 2003 and 1988. Corn containing greater than 500 ppb aflatoxin cannot be blended.
- IA mandating aflatoxin testing in milk for M1
- *Aspergillus* most present in 2012
- Hot/Dry growing season provides climate for the mold *Fusarium verticillioides*, which causes fumonisin B1.

Why is Aflatoxin of concern?

 U.S. Department of Health & Human Services

 **U.S. Food and Drug Administration**
Protecting and Promoting *Your* Health

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Recall -- Firm Press Release

FDA posts press releases and other notices of recalls and market withdrawals from the firms involved as a service to consumers, the media, and other interested parties. FDA does not endorse either the product or the company.

Diamond Pet Food Recalled Due to Aflatoxin

Contact:
Mark Brinkman
573-229-4203

FOR IMMEDIATE RELEASE -- December 20, 2005 -- Diamond Pet Food has discovered aflatoxin in a product manufactured at our facility in Gaston, South Carolina. Aflatoxin is a naturally occurring toxic chemical by-product from the growth of the fungus *Aspergillus flavus*, on corn and other crops.

Out of an abundance of caution, we have notified our distributors and recommended they hold the sale of all Diamond Pet Food products formulated with corn that were produced out of our Gaston facility (see complete list below). Please note that products manufactured at our facilities in Meta, Missouri and Lathrop, California are not affected. The Gaston facility date codes are unique from other Diamond facility codes in that either the eleventh or twelfth character in the date code will be a capital "G" (in reference to Gaston). The range of date codes being reviewed are "Best By 01-March-07" through "Best By 11-June-07". Diamond's quantitative analysis records substantiate that Diamond's corn shipments were definitively clear of aflatoxin after December 10. As such, "Best By 11-June-07" date codes or later are not affected by this notice.

States serviced by our Gaston facility include Alabama, Connecticut, Delaware, Florida, Georgia, Kentucky (eastern), Maine, Maryland, Massachusetts, Mississippi, New Hampshire, New Jersey, New York, North Carolina, Ohio, Pennsylvania, Rhode Island, South Carolina, Tennessee, West Virginia, Vermont, and Virginia.

We are rapidly analyzing retained samples of all products produced in Gaston so we can isolate specific lot numbers impacted and provide this information to our distributors, retailers and customers as quickly as possible.

Meanwhile, if your pet is showing any symptoms of illness, including sluggishness or lethargy combined with a reluctance to eat, yellowish tint to the eyes and/or gums, and severe or bloody diarrhea, please consult your veterinarian immediately.

Product quality and customer satisfaction are important to us. We pledge to keep you updated as new developments occur.

Gaston Facility Products Removed From Sale

Diamond Low Fat Dog Food
Diamond Hi-Energy Dog Food
Diamond Maintenance Dog Food
Diamond Performance Dog Food
Diamond Premium Adult Dog Food
Diamond Puppy Food
Diamond Maintenance Cat Food
Diamond Professional Cat Food
Country Value Puppy
Country Value Adult Dog
Country Value High Energy Dog
Country Value Adult Cat Food
Professional Chicken & Rice Senior Dog Food
Professional Reduced Fat Chicken & Rice Dog Food
Professional Adult Dog Food
Professional Large-Breed Puppy Food
Professional Puppy Food
Professional Reduced Fat Cat Food
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How to Monitor or Test for Aflatoxins?

Mycotoxin Regulations

- Divided between human consumption and animal feed
- EU has some regulations with each country then elaborates or adds to the “base” regulations
- US has regulations and advisory or guidance levels
- Over 100 countries have mycotoxin regulations

Action Levels of Aflatoxins: USA

Must Not Exceed	Description
20 ppb	For corn and other grains intended for immature animals (including immature poultry) and for dairy animals , or when it's destination is not known
20 ppb	For animal feeds, other than corn or cottonseed meal
100 ppb	For corn and other grains intended for breeding beef cattle, breeding swine, or mature poultry
200 ppb	For corn and other grains intended for finishing swine of 100 pounds or greater
300 ppb	For corn and other grains intended for finishing (i.e., feedlot) beef cattle and for cottonseed meal intended for beef cattle, swine or poultry
20 ppb	Food Commodities for human consumption
0.5 ppb (Aflatoxin M1)	Milk

Action Levels of Aflatoxins: EUROPE

Aflatoxin in cereals and processed products there of intended for direct human consumption or use as an ingredient in foodstuffs	2 ppb Aflatoxin B1 4 ppb total
Aflatoxin in milk	0.05 ppb

CODEX Level = 0.5 ppb

Current Practices for Control of Mycotoxins

Grains and Feeds

- Climate and weather- health of plant
- Mycotoxins monitored with quick high throughput tests
- Feed and grains are not 100% tested
- Mycotoxins not uniformly distributed and samplings are small relative to the batch size
- UV light not 100% effective interfered by competitive mold strains
- Binders reduce nutrient value

Milk

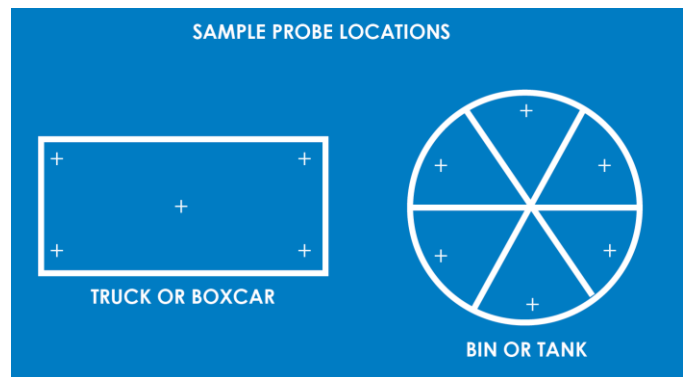
- Milk, with regard to aflatoxin, is a uniform sample of the farm environment and geographic regions
- Milk is a reflective of a large sample of feed/grain
- Used to monitor and verify feed and grain controls for aflatoxins are working
- Screening of milk is advised in regions with elevated feed/grain risk
- **Raw milk qualification is primary control for all dairy products**

Challenges in testing for Mycotoxins

- Mycotoxins are not evenly distributed in feeds or grains
- Grain from individual plants or from individual seeds/kernels from one plant, may be highly contaminated
- Grain from other plants, or other seeds/kernels from those same plant may be mycotoxin-free



Sampling



Sample Size	Kernels (estimate)	Variability (ppb)
10 lbs	30,000	11.6-28.4
5.0 lbs	15,000	8.1-31.9
2.5 lbs	7,500	3.2-38.8
1.0 lbs	3,000	1-46.9

- The objective of sample collection is to collect a specimen from as many parts of the grain or feed pile as possible.
- The size of the collected specimen depends on the size of the load or storage bin in which the grain or feed is located.
- For a typical truck or wagon-load size of grain/ feed, collect at least 3 lbs
- For a train-car load size of grain or feed, collect at least 10 pounds

(Source: NE Cooperative Extension)

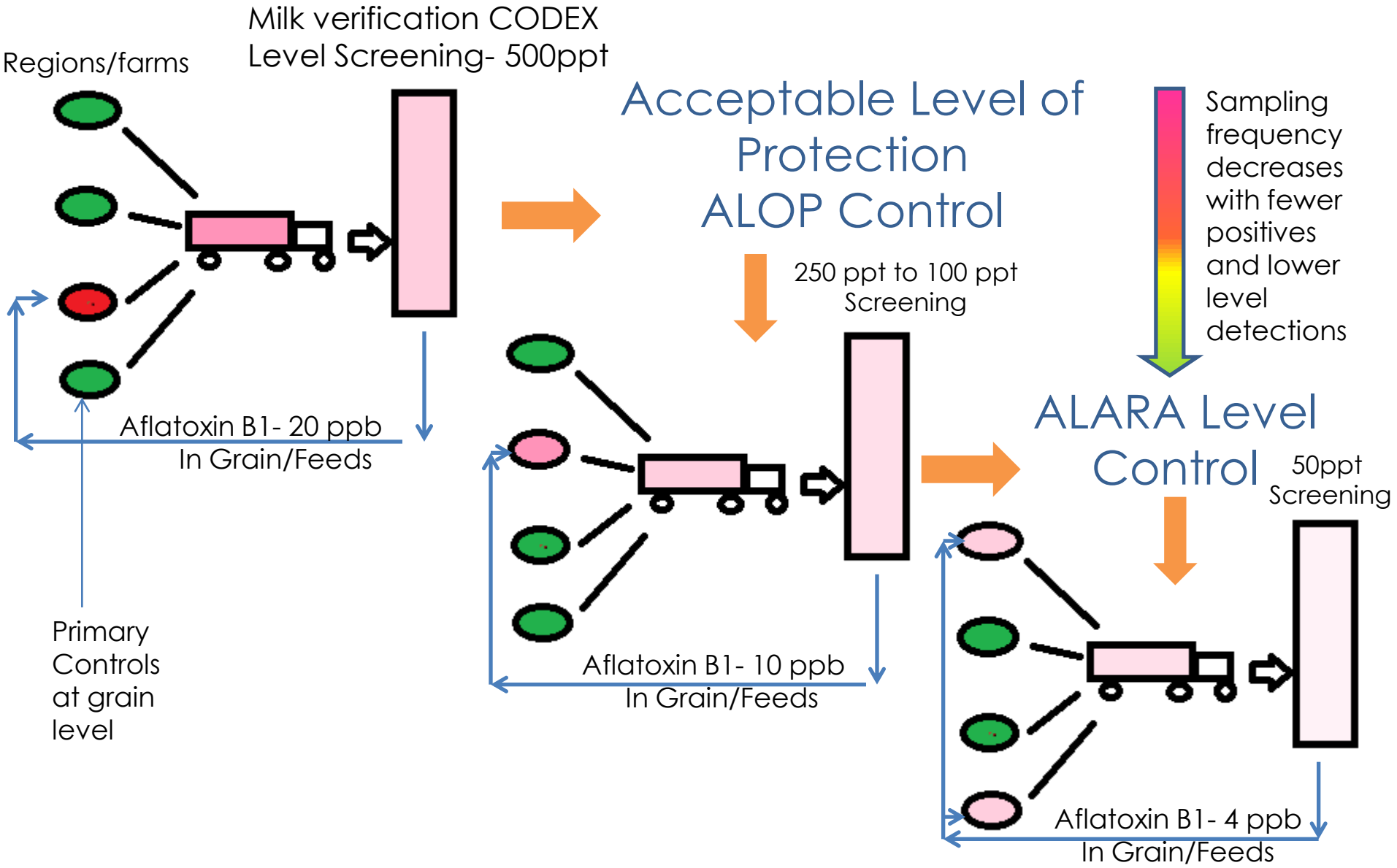
Example of US control system

- Monitoring corn growth sends advisory that conditions favorable for aflatoxin production
- Feed/grain mills prepare pre-harvest for testing with a higher sampling frequency
- High frequency of positive corn reported
- Public health advisory to dairies to screen all milk
- Dairies incorporate Aflatoxin M1 with antibiotic testing of truck. Milk positive at 500 ppt is rejected.
- Problem farms and regions remediated.
- Advisory lifted after 6 months and low incidence
- Dairies adopt a periodic screening check of milk

Example of European Control

- Milk in Eastern Europe detected positive with scheduled regulatory monitoring at Maximum level 50 ppt- ALARA (As Low As Reasonably Achievable)
- Milk is tested and reported safe at CODEX level
- Farm trace back and removal of feeds from food supply based on EU grain level 2 ppb.
- ALARA in milk supply re-established within 30 days.
- Resume scheduled regulatory monitoring
- Dairies adopt routine methods for periodic screening

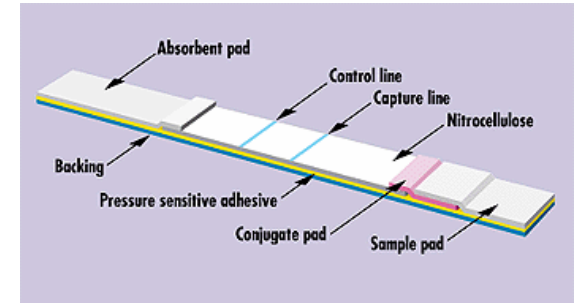
Integrated Grain and Milk Aflatoxin Management and Continuous Improvement



Available Testing Method Choices

Ease of Use

Lateral Flow

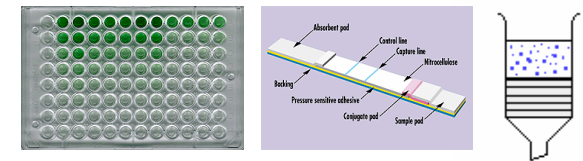


Rapid Methods

ELISA

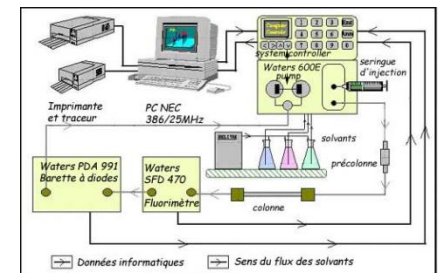
Lateral Flow

Immunoaffinity Column



Broadest Applications

HPLC

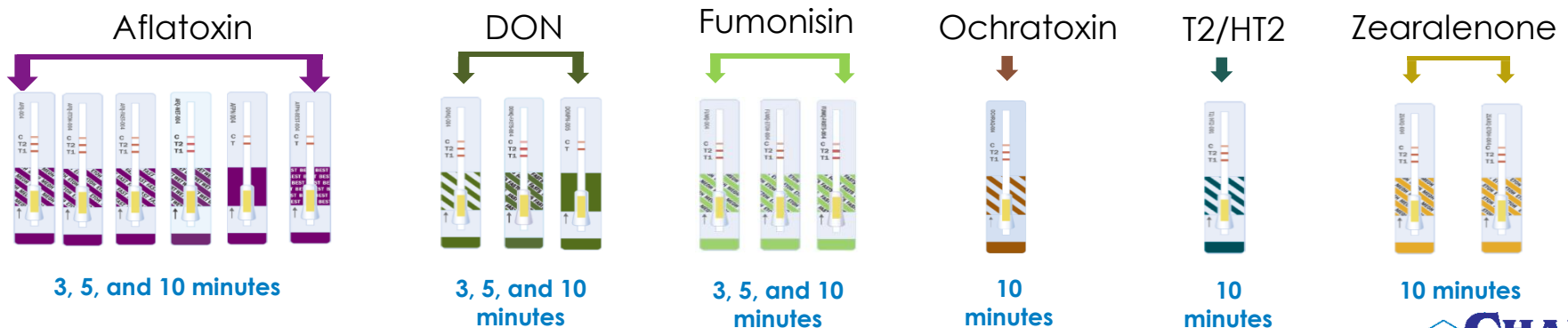


Grain Screening Methods

In US methods are validated by USDA – Grain Inspection Packers and Stockyard Association – GIPSA- Methods updated and re-validated frequently- http://www.gipsa.usda.gov/fgis/tech-servsup/metheqp/GIPSA_Approved_Mycotoxin_Rapid_Test_Kits.pdf



- Trend of method development is fast single use tests- lateral flow tests are achieving sensitivities of ELISA and HPLC
- New improvements are using non-organic solvents for extraction making earlier on-farm and truck-side testing easier
- Screening is being done in 3 to 10 minutes



How to Monitor or Test for Aflatoxins?

Charm ROSA SLAFMQ = screen at US Safe Level 0.5 ppb

Charm ROSA MRLAFMQ = screen at EU MRL 0.05 ppb



Summary

- Mycotoxins - where does it come from?
 - Fungus contamination on grains in field or post harvest
- What is Aflatoxin?
 - Toxin produced by this fungus
- Why is Aflatoxin of concern?
 - Dangerous carcinogen to humans and animals
- How to monitor or test for Aflatoxin?
 - Screening of grain and milk during periods of concern



Thank You

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