AGENDA

• Tim Birmingham, Almond Board of California, moderator
• Tom Whitaker, NC State / USDA
The Aflatoxin Menace
- Addressing Head on -

Tom Whitaker
USDA/ARS Retired
Professor Emeritus
NC State University
Aspergillus flavus
Almonds
US - 20 T

Canada
15 T

Japan
10 T

EU
10 T / 8 B1

Mexico
20 T

30%
4%
50%
12%
US Exporter

Customer Importer

Aflatoxin Concentration = ?
ABC Staff & Industry Have Been Proactive in Developing Programs that Produce Information to Better Manage Aflatoxin
Aflatoxin Studies

1) Aflatoxin Risk Categories
2) Sorting Efficiency
3) Sample Accuracy and Precision
4) Codex Aflatoxin Standard MLs/Samp Plans/Tree nut
5) B1/Total Aflatoxins Ratio
6) VASP/PEC – EU
7) Aflatoxin by Product Category
8) Method to Reduce Lots Rejected at Destination
Aflatoxin Risk Categories
Where is the Aflatoxin?

- Poor vs good quality nuts
- Grade factors
# Average of 50 Lots

<table>
<thead>
<tr>
<th>Grade Category</th>
<th>Weight (%)</th>
<th>Aflatoxin (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High Quality</td>
<td>83.7</td>
<td>3.2</td>
</tr>
<tr>
<td>Mechanical</td>
<td>7.4</td>
<td>7.9</td>
</tr>
<tr>
<td><strong>Insect</strong></td>
<td><strong>7.2</strong></td>
<td><strong>76.3</strong></td>
</tr>
<tr>
<td>Other defect</td>
<td>1.5</td>
<td>11.8</td>
</tr>
<tr>
<td>Mold</td>
<td>0.2</td>
<td>0.8</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>100.0</strong></td>
<td><strong>100.0</strong></td>
</tr>
</tbody>
</table>
## Average of 50 Lots

<table>
<thead>
<tr>
<th>Grade Category</th>
<th>Weight (%)</th>
<th>Aflatoxin (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High Quality</td>
<td>83.7</td>
<td>3.2</td>
</tr>
<tr>
<td>Poor Quality</td>
<td>16.3</td>
<td>96.8</td>
</tr>
<tr>
<td>Total</td>
<td>100.0</td>
<td>100.0</td>
</tr>
</tbody>
</table>
Efficiency of Electronic and Hand Sorting at Removing Aflatoxin Contaminated Almonds
<table>
<thead>
<tr>
<th>Crop Year</th>
<th>Lots Tested</th>
<th>Aflat. (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2007</td>
<td>15,022</td>
<td>0.48</td>
</tr>
<tr>
<td>2008</td>
<td>13,208</td>
<td>0.45</td>
</tr>
<tr>
<td>2009</td>
<td>10,007</td>
<td>0.59</td>
</tr>
<tr>
<td>2010</td>
<td>12,611</td>
<td>0.32</td>
</tr>
<tr>
<td>2011</td>
<td>13,580</td>
<td>0.27</td>
</tr>
<tr>
<td>2012</td>
<td>13,700</td>
<td>0.38</td>
</tr>
<tr>
<td>2013</td>
<td>15,028</td>
<td>0.33</td>
</tr>
</tbody>
</table>
VASP/PEC

Industry-wide export aflatoxin sampling program to reduce the number of U.S. lots rejected by the EU
## US Export Aflatoxin Sampling Plan (VASP) RTE Almond Lots

<table>
<thead>
<tr>
<th>Year</th>
<th>U.S. Plan</th>
<th>EU Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>2007-10</td>
<td>3x5 kg ≤ 2T</td>
<td>2B1/4T</td>
</tr>
</tbody>
</table>

EU Plan before March 2010 - 3x10 kg ≤ 2B1/4T
Codex Standard for Tree Nuts
ML & Sampling Plan (2010)

RTE: 2x10kg < 10 ppb T
DFP: 1x20kg < 15 ppb T

T = Total aflatoxins - No B1
EU adds B1 ML to Codex Std

Ratio B1/Total Aflatoxin
Total=B1+B2+G1+G2
B1/Total Ratio

- Samples Tested – 2,656 (T >0.5 ppb)
- **Mean Ratio (%)** - 86.4
- **Median Ratio (%)** - 100.0
- **Distribution** - Negatively Skewed
# US Export Aflatoxin Sampling Plan (VASP) RTE Almond Lots

<table>
<thead>
<tr>
<th>Year</th>
<th>U.S. Plan</th>
<th>EU Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 2010</td>
<td>3x5 kg ≤ 2T</td>
<td>2B1/4T</td>
</tr>
<tr>
<td>&gt;2010</td>
<td>2x10 kg ≤ 8B1/10T</td>
<td>8B1/10T</td>
</tr>
</tbody>
</table>

EU Before March 2010 - 3x10 kg ≤ 2B1/4T  
EU After March 2010 - 2x10 kg ≤ 8B1/10T
## VASP % Lots Accepted & Rejected

<table>
<thead>
<tr>
<th>Crop Year</th>
<th>Lots Tested</th>
<th>Accept (%)</th>
<th>Reject (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2007</td>
<td>15,022</td>
<td>94.77</td>
<td>5.23</td>
</tr>
<tr>
<td>2008</td>
<td>13,208</td>
<td>95.33</td>
<td>4.67</td>
</tr>
<tr>
<td>2009</td>
<td>10,007</td>
<td>94.87</td>
<td>5.13</td>
</tr>
<tr>
<td>2010</td>
<td>12,611</td>
<td>98.72</td>
<td>1.29</td>
</tr>
<tr>
<td>2011</td>
<td>13,580</td>
<td>98.88</td>
<td>1.12</td>
</tr>
<tr>
<td>2012</td>
<td>13,700</td>
<td>98.41</td>
<td>1.59</td>
</tr>
<tr>
<td>2013</td>
<td>15,028</td>
<td>98.76</td>
<td>1.24</td>
</tr>
</tbody>
</table>
Sampling Studies

How accurately and precisely does sample ppb estimate the true lot ppb?
Lot (20,000 kg) ppb = ?

Sample (10 kg) ppb

- Lot ppb = Sample ppb ?
- Sample ppb ≤ Regulatory Limit
Lot 10
Mean = 22
Median = 3
CV = 138%
Lot

Sample

Sample Preparation

Mill

Test Procedure

Analysis

Sample Test Result
## Almond Lot Aflatoxin = 10 ppb

<table>
<thead>
<tr>
<th>Test Procedure</th>
<th>Size</th>
<th>Variance</th>
<th>Ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample-kg</td>
<td>10</td>
<td>209.6</td>
<td>94.4</td>
</tr>
<tr>
<td>Test Port.-g</td>
<td>100</td>
<td>12.0</td>
<td>5.4</td>
</tr>
<tr>
<td>HPLC-Aliq</td>
<td>1</td>
<td>0.4</td>
<td>0.2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>222.0</strong></td>
<td><strong>100.0</strong></td>
<td><strong>100.0</strong></td>
</tr>
</tbody>
</table>
Variability Leads to Misclassification of Lots

• Good lots rejected
  – (Exporter’s risk)
• Bad lots accepted
  – (Importer’s risk)
Lot 10
Mean = 22
Median = 3
CV = 138%

16x10 kg Almonds Samples
Sampling Plan Evaluation Method (OC Development)

- Test Procedure
- Variability Distribution Theory
- Seller’s Risk
- Buyer’s Risk
- Total Lots Rej
- Mytox. Removed

Acc/Rej Limit (Reg Limit)
EU Plan-Shelled Almonds

- Tolerance
- $3 \times 10^3 \text{ kg} \leq 4T/2B1$
- $2 \times 10^2 \leq 8B1/10T$

Lot Aflatoxin Concentration (ng/g)
Probability of Accept (%)

Lot Aflatoxin Concentration (ng/g)

3x10 kg $\leq 4T/2B1$
2x10 kg $\leq 8B1/10T$

EU Plan - Shelled Almonds

- $3 \times 10^3 \text{ kg} \leq 2B1/4T$
- $2 \times 10^2 \leq 8B1/10T$

Probability of Accept (%) vs. Lot Aflatoxin Concentration (ng/g)
FAO Mycotoxin Sampling Tool
(Food Safety Risk Analysis Tools)

http://www.fstools.org/mycotoxins/

*Based upon USDA/ARS Model*
Develop a method to predict the number of U.S. almond lots rejected by the EU at destination

Rejects reflect:
1) Aflatoxin level in lots tested in US
2) Design of US export sampling plan
3) Design of EU import sampling plan
Objectives

1) Determine effect of U.S. aflatoxin levels and PEC sampling plan on lot rejected in the EU

2) Using PEC sample test results, predict at time of U.S. testing the % US lots that will be rejected at a later date in the EU

3) Modify PEC sampling plan to keep U.S. lots rejected in the EU to acceptable levels
Aflatoxin Distribution Among Lots At Origin

U.S. Aflatoxin Export Sampling Plan (100%)

Lots Accepted for Export To EU

EU Aflatoxin Sampling Plan (1-10% Testing Rate)

Lots Accepted At Destination

Lots Rejected At Origin

Lots Rejected At Destination
Lot Aflatoxin Concentration (ng/g AFT)

Cumulative Freq. Dist. (%)

- 2008 crop – 0.45 ng/g AFT
- 2006 crop – 1.26 ng/g AFT
- Hypothetical crop – 3.00 ng/g AFT
- Hypothetical crop – 6.00 ng/g AFT
<table>
<thead>
<tr>
<th>US1/EU</th>
<th>2x10 kg ≤ 8B1/10T</th>
</tr>
</thead>
<tbody>
<tr>
<td>US2</td>
<td>2x10 kg ≤ 8T</td>
</tr>
<tr>
<td>US3</td>
<td>2x10 kg ≤ 6T</td>
</tr>
<tr>
<td>US4</td>
<td>2x10 kg ≤ 4T</td>
</tr>
<tr>
<td>US5</td>
<td>2x10 kg ≤ 2T</td>
</tr>
<tr>
<td>US6</td>
<td>2x10 kg ≤ 0.5T</td>
</tr>
<tr>
<td>US Plans 2x10 kg</td>
<td>US Lot Contamination (ppb)</td>
</tr>
<tr>
<td>------------------</td>
<td>-----------------------------</td>
</tr>
<tr>
<td></td>
<td>0.42</td>
</tr>
<tr>
<td>8B1 / 10T</td>
<td></td>
</tr>
<tr>
<td>8T</td>
<td></td>
</tr>
<tr>
<td>6T</td>
<td></td>
</tr>
<tr>
<td>4T</td>
<td></td>
</tr>
<tr>
<td>2T</td>
<td></td>
</tr>
<tr>
<td>0.5T</td>
<td></td>
</tr>
</tbody>
</table>

- % lots acc/rej in US
- Avg. AFT in acc/rej lots in US
- % lots acc/rej in EU
- Avg. AF in acc/rej lots in EU
Aflatoxin Distribution Among Lots
11,465 (1.26 ppb)

PEC Sampling Plan (100%)
2 x 10 kg ≤ 8B1 / 10T

Lots Accepted for Export
11,000 (0.38 ppb)

Lots Rejected (4.1%)
465 (23.82 ppb)

EU Sampling Plan (5%=550)
2 x 10 kg ≤ 8B1 / 10T

Lots Accepted
10,991 (0.25 ppb)

Lots Rejected (9 lots)
1.54% (8.89 ppb)
<table>
<thead>
<tr>
<th>US Aflat Level (ppb)</th>
<th>US Rej (%)</th>
<th>Avg Aflat in Exports (ppb)</th>
<th>Rej in EU (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.42</td>
<td>1.36</td>
<td>0.15</td>
<td>0.51</td>
</tr>
<tr>
<td>1.26</td>
<td>4.06</td>
<td>0.38</td>
<td>1.54</td>
</tr>
<tr>
<td>3.00</td>
<td>9.31</td>
<td>0.77</td>
<td>3.56</td>
</tr>
<tr>
<td>6.00</td>
<td>17.66</td>
<td>1.44</td>
<td>6.94</td>
</tr>
</tbody>
</table>

US=2x10kg<8B1/10T
All Almond Crops and VASP
100% US Testing
EU Testing 2x10 kg ≤ 8B1/10T
<table>
<thead>
<tr>
<th>US Aflat Level (ppb)</th>
<th>US Rej (%)</th>
<th>Avg Aflat in Exports (ppb)</th>
<th>Rej in EU (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.42</td>
<td>1.36</td>
<td>0.15</td>
<td>0.51</td>
</tr>
<tr>
<td>1.26</td>
<td>4.06</td>
<td>0.38</td>
<td>1.54</td>
</tr>
<tr>
<td>3.00</td>
<td>9.31</td>
<td>0.77</td>
<td>3.56</td>
</tr>
<tr>
<td>6.00</td>
<td>17.66</td>
<td>1.44</td>
<td>6.94</td>
</tr>
</tbody>
</table>

US=2x10kg<8B1/10T
Rej in EU (%) = 0.39 * Rej in US (%)

US using 2x10kg<8B1/10T
## US Lots Rejected in EU (%)

<table>
<thead>
<tr>
<th>US Plans 2x10 kg</th>
<th>US Lot Contamination (ng/g)</th>
<th>0.42</th>
<th>1.26</th>
<th>3.00</th>
<th>6.00</th>
</tr>
</thead>
<tbody>
<tr>
<td>8B1 / 10T</td>
<td></td>
<td>0.51</td>
<td>1.54</td>
<td>3.56</td>
<td>6.94</td>
</tr>
<tr>
<td>8T</td>
<td></td>
<td>0.48</td>
<td>1.46</td>
<td>3.38</td>
<td>6.54</td>
</tr>
<tr>
<td>6T</td>
<td></td>
<td>0.42</td>
<td>1.26</td>
<td>2.92</td>
<td>5.74</td>
</tr>
<tr>
<td>4T</td>
<td></td>
<td>0.34</td>
<td>1.03</td>
<td>2.38</td>
<td>4.71</td>
</tr>
<tr>
<td>2T</td>
<td></td>
<td>0.24</td>
<td>0.73</td>
<td>1.69</td>
<td>3.37</td>
</tr>
<tr>
<td>0.5T</td>
<td></td>
<td>0.13</td>
<td>0.40</td>
<td>0.91</td>
<td>1.85</td>
</tr>
</tbody>
</table>
Aflatoxin Distribution Among Lots At Origin

U.S. Aflatoxin Export Sampling Plan (100%)

Lots Accepted for Export To EU

EU Aflatoxin Sampling Plan 2x10 kg ≤ 8B1/10T

Lots Accepted At Destination

Lots Rejected At Origin

Lots Rejected At Destination-RAFF
US Testing Plan 2x10 kg, A/R Level Shown
100% US Testing
EU 2x10 kg ≤ 8B1/10T

2x10 kg ≤ 8B1/10T
2x10 kg ≤ 8T
2x10 kg ≤ 6T
2x10 kg ≤ 4T
2x10 kg ≤ 2T
2x10 kg ≤ 0.5T

Lot Rejected in US (%)
Lots Rejected in EU (%)

US Testing Plan 2x10 kg, A/R Level Shown
100% US Testing
EU 2x10 kg ≤ 8B1/10T

2x10 kg ≤ 8B1/10T
2x10 kg ≤ 8T
2x10 kg ≤ 6T
2x10 kg ≤ 4T
2x10 kg ≤ 2T
2x10 kg ≤ 0.5T

Lot Rejected in US (%)
Lots Rejected in EU (%)

US Testing Plan 2x10 kg, A/R Level Shown
100% US Testing
EU 2x10 kg ≤ 8B1/10T

2x10 kg ≤ 8B1/10T
2x10 kg ≤ 8T
2x10 kg ≤ 6T
2x10 kg ≤ 4T
2x10 kg ≤ 2T
2x10 kg ≤ 0.5T

Lot Rejected in US (%)
Lots Rejected in EU (%)

US Testing Plan 2x10 kg, A/R Level Shown
100% US Testing
EU 2x10 kg ≤ 8B1/10T

2x10 kg ≤ 8B1/10T
2x10 kg ≤ 8T
2x10 kg ≤ 6T
2x10 kg ≤ 4T
2x10 kg ≤ 2T
2x10 kg ≤ 0.5T

Lot Rejected in US (%)
Lots Rejected in EU (%)

US Testing Plan 2x10 kg, A/R Level Shown
100% US Testing
EU 2x10 kg ≤ 8B1/10T

2x10 kg ≤ 8B1/10T
2x10 kg ≤ 8T
2x10 kg ≤ 6T
2x10 kg ≤ 4T
2x10 kg ≤ 2T
2x10 kg ≤ 0.5T

Lot Rejected in US (%)
Lots Rejected in EU (%)
<table>
<thead>
<tr>
<th>Crop (CY)</th>
<th># Lots Tested</th>
<th># Lots Accepted</th>
<th># Lots Failed</th>
<th>% Failed</th>
<th>% Accept</th>
<th>Avg. Afla (all lots tested)</th>
<th>Avg. Afla (Failed Lots)</th>
<th>Avg. Afla (Accepted Lots)</th>
<th>% Insect Damage</th>
</tr>
</thead>
<tbody>
<tr>
<td>2015 (15/16)</td>
<td>13870</td>
<td>13644</td>
<td>226</td>
<td>1.63%</td>
<td>98.37%</td>
<td>0.63</td>
<td>19.89</td>
<td>0.24</td>
<td>1.30%</td>
</tr>
<tr>
<td>2016 (16/17)</td>
<td>14648</td>
<td>14387</td>
<td>261</td>
<td>1.78%</td>
<td>98.22%</td>
<td>0.59</td>
<td>17.68</td>
<td>0.24</td>
<td>1.20%</td>
</tr>
<tr>
<td>2017 (17/18)</td>
<td>15224</td>
<td>14263</td>
<td>961</td>
<td>6.31%</td>
<td>93.69%</td>
<td>1.48</td>
<td>17.73</td>
<td>0.35</td>
<td>2.40%</td>
</tr>
<tr>
<td>2018 (18/19)</td>
<td>1499</td>
<td>1409</td>
<td>90</td>
<td>6.00%</td>
<td>94.00%</td>
<td>1.05</td>
<td>13.09</td>
<td>0.29</td>
<td>1.70%</td>
</tr>
</tbody>
</table>

2015 to 2017 -> 2x10kg<8B1/10T
2018 -> 2x10kg<5T
<table>
<thead>
<tr>
<th>US Aflat Level (ppb)</th>
<th>US Rej (%)</th>
<th>Avg Aflat in Exports (ppb)</th>
<th>Rej in EU (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.42</td>
<td>1.36</td>
<td>0.15</td>
<td>0.51</td>
</tr>
<tr>
<td>1.26</td>
<td>4.06</td>
<td>0.38</td>
<td>1.54</td>
</tr>
<tr>
<td>3.00</td>
<td>9.31</td>
<td>0.77</td>
<td>3.56</td>
</tr>
<tr>
<td>6.00</td>
<td>17.66</td>
<td>1.44</td>
<td>6.94</td>
</tr>
</tbody>
</table>

US=2x10kg<8B1/10T
Thank you!