PACA COUNTRY ACTIVITIES: rationale and approaches
Country Activities – PACA STRATEGY

9 - Partnership for Aflatoxin Control in Africa (PACA) Mid-Term Strategic Plan, 2014-2017

1. Introduction

In April 2012, a PACA Strategy Development - Stakeholder Consultative Workshop was convened in Durban, South Africa. The Strategy Development process was led by broad and inclusive representation of all major stakeholders from health, trade and agriculture sectors. Based on discussions during the workshop, a PACA strategy was developed. The PACA Strategy 2013-2022 will guide PACA efforts for the next ten years.

The ultimate goal of PACA is to protect human health by minimizing aflatoxin exposure and to contribute to a prosperous Africa through overcoming the multipurpose economic and developmental implications of the aflatoxin problem.

The PACA Team believes this was achieved by ensuring the PACA Strategy, 2013-2022 into strategic actions for 2014-2017. It was not written outside of a consultative process with all stakeholders and key binding partners (KBP) as outlined in the PACA Strategy 2013-2022, i.e. a multi-stakeholder, multi-faceted, multi-dimensional strategy to be implemented by PACA with the support of partners and stakeholders to ensure implementation.

2. PACA Strategy Thematic Areas and Key Result Areas

The analysis of causes, drivers, epizootiology, and challenges in the PACA Strategy 2013-2022 identified five complementary thematic areas. Together these define the main areas that PACA will address during the next decade to deliver on its mission to support agricultural development, improve consumer health, and facilitate trade by catalyzing, coordinating, and increasing effective aflatoxin control along agricultural value chains in Africa.

The five PACA strategic thematic areas and key result areas (KRBs) are shown below.

Thematic Areas and Key Result Areas (KRBs) are shown below.

Thematic Areas

1. Country-Aflatoxin Research and Policy
   - KRB 1.1. Generating information and evidence to inform interventions
   - KRB 1.2. Facilitating adaptation and scale-up of sustainable technologies and knowledge
   - KRB 1.3. Developing new technologies and knowledge to improve aflatoxin prevention and control
   - KRB 1.4. Enhancing research and research facilities

2. Policy, Legislation, and Trade Policy for the Management of Aflatoxins
   - KRB 2.1. Enhancing policy analysis and formulation
Country Activities: Development of PACA Strategy

- Consultative
- Multi-stakeholder
- Multi-Sectoral
- Inclusive
- Transparent

PACA Strategy Development - Stakeholder Consultation Workshop
Dar es Salaam, Tanzania, 10-12 April, 2013.
**Country Activities:** as Identified by PACA SC

1. Establish **Africa Aflatoxin Information Management System (AfricaAIMS)**

2. Support country-led aflatoxin situation analysis and action planning;

3. Mainstream aflatoxin control through the PACA initiative in CAADP National Agriculture and Food Security Investment Plans (NAFSIPs).
Country Activities

AfricaAIMS

• Major challenge in mitigating aflatoxin = lack of adequate information and harmonized data collection on the subject.

• Yet information is needed to:
  • Inform policy and interventions;
  • Inform prioritization of resource allocation;
  • Promote country capacity and awareness on the aflatoxin issue in Africa
Country Activities

Country Led Situation Analysis

• Country-led food safety system and aflatoxin situation analysis and action planning will:

• Create empirical to inform interventions

• Examples of Information to be collected:
  • *existing aflatoxin prevalence*,
  • *economic data due to aflatoxin contamination*
  • *legislation*,
  • *policy and regulation*,
Country Activities

Mainstreaming Aflatoxin Mitigation into NAFSIPS

• Using results from situation analysis, PACA will support Countries in developing Comprehensive Aflatoxin Strategy and Investment Programme to be mainstreamed into NAFSIPs. By so doing, countries will:

  • Ease the process of identifying gaps for aflatoxin interventions in country policies;
  • Increase national investment in aflatoxin issues and attract other funds for country aflatoxin initiatives;
  • Facilitate the alignment of aflatoxin interventions and projects with country priorities through NAFSIPs;
  • Ensure that political leadership and ownership of aflatoxin issues at country level is established.
PACA support to pilot countries

1. Engaging consultants for country-led situation analysis

2. Aflatoxin testing facilities and training

3. Support in mainstreaming aflatoxin control
Main messages

• Governments play central role in aflatoxin control

• There is little support to governments to play that role effectively

• PACA arms at providing support to countries
PACA in-country training

Aflatoxin analysis in food and feed

September 2014
• **Food security and food safety**

  – **Food Security** exists when all people, at all times, have physical and economic access to **sufficient**, **safe** and **nutritious** food to meet their dietary needs and food preferences for an active (**productive**) and **healthy life** (World Food Summit, 1996. In: FAO 2006. Policy Brief).

• Factors that affect food safety
  – Presence of physical materials
• Aspergillus spp, Fusarium spp and Penicillium spp (Pitt, 2000, Med. Mycol. 38: 17-22)
• **Physical aspects of infected food item**
  
  – Discoloration
  
  – Moldy taste
  
  – Production of off odor
  
  – Production of mycotoxin: aflatoxin
Conidia and sclerotia serve as infective propagules of *Aspergillus* species in soil.

Propagules *Aspergillus* can contaminate various crops directly (direct contact) or indirectly (dispersal by wind or insect).
Ecological parameters affecting mycotoxin production

- Moisture
- Spore Load
- Temperature
- Competing Microflora
- Substrate
- CO$_2$/O$_2$
- Time
- Mechanical Damage
- Insect Damage
- Drought Stress
Aspergillus flavus
A. parasiticus (Kurtzman et al., 1987)
A. nomius (Wilson et al., 2002)
A. pseudotamarii (Ito et al., 2001)
A. bombycis (Peterson et al., 2001)

Fusarium spp

Penicillium spp

Aflatoxins, Cyclopiazonic acid

Fumonisins

Ochratoxin, Citrinin
Background (cont.)

Mycotoxins

• Mykes: Greek for fungus/mold

• Toxicum: Latin for poison/toxin
Mycotoxins of economic, health and agricultural significance

- **Mycotoxins** are metabolic products of food spoilage fungi that induce toxic responses when consumed by animals or people.

- **Hundreds** of mycotoxins have been identified; They will fall into many different chemical classes, and induce a wide variety of toxic responses.
Background (cont.)

- **~4.5 billion** people are chronically exposed (WHO, 2004)

- **Health hazards (human)**
  
  - **Acute toxicity**
    
    - Death (Lewis et al., 2005, Environ. Health Pers. 113: 1763-1767)
    
    - **High Doses:** Lethal (125 deaths in 2004 in Kenya with levels up to 8,000 ppb) (Lewis et al., 2005); 9 deaths, June 2, 2014 (CDC, 2014)

  - **Chronic toxicity**
    
    - Hepatocarcinogen (IARC, 1993); liver cirrhosis (IARC, 1993; 2012)
    
    

- **Impact on trade**
  
  - 25% global food supply contaminated (FAO, 2000)
  
  - 64% reduction in food quality in Africa (WHO, 2001)
Background (cont.)

- **Mycotoxin Production**
  - **Harmful to animal**
    - Poultry: feed intake, reduce eggs production, carryover in eggs and meat and death (Otim Maxwell et al., 2005 Avian Pathol. 34, 319-323)

Fed with aflatoxin “free” maize grain
Fed with aflatoxin contaminated maize grain
Effects of aflatoxins

Livers from guinea pigs given increasing doses of aflatoxins over the same period of time. From left to right beginning in the upper left corner to the lower right corner with a liver from a guinea pig given the greatest dose of aflatoxins. Note the increasingly pale livers with increasing dose of aflatoxins.
Background (cont.)

Worldwide regulation of Mycotoxins

Worldwide Mycotoxin Regulations Tool

www.commodityregs.com
Background (cont.)
Web Based or Mobile App (iOS or Android)
Choose Your Sample...

<table>
<thead>
<tr>
<th>Commodity</th>
<th>Commodity</th>
<th>Commodity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barley</td>
<td>Beer</td>
<td>Botanicals</td>
</tr>
<tr>
<td>Cocoa</td>
<td>Coffee</td>
<td>Confectionery</td>
</tr>
<tr>
<td>Copra</td>
<td>Corn/Maize</td>
<td>Cottonseed</td>
</tr>
<tr>
<td>Dairy</td>
<td>Dried Fruit</td>
<td>Feeds</td>
</tr>
<tr>
<td>Fresh Fruit/Juice</td>
<td>Groundnuts/Peanuts</td>
<td>Infant Formula</td>
</tr>
<tr>
<td>Oats</td>
<td>Rice</td>
<td>Rye</td>
</tr>
<tr>
<td>Sorghum</td>
<td>Soy</td>
<td>Spices</td>
</tr>
<tr>
<td>Tree Nuts</td>
<td>Wheat</td>
<td>Wine</td>
</tr>
<tr>
<td>All Commodities</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Background (cont.)

Get the Details...

Regulations for Groundnuts/Peanuts in United States [source]

**Maximum Allowable/Recommended Units**

Total aflatoxins: 20 ppb

Selected Molds That Produce Toxins

Aspergillus flavus, Aspergillus parasiticus

Commodities Susceptible to Contamination

Barley, cocoa, coffee, confectionery, corn/maize, copra, dairy, dried fruit, feeds, groundnuts/peanuts, infant formula, oats, rice, rye, sorghum, soy, spices, tree nuts, and wheat

Health Effects

Liver cancer and damage
Immunosuppression
Decreased milk and egg production

VICAM Tests/Testing Ranges

<table>
<thead>
<tr>
<th>Test</th>
<th>Method</th>
<th>Cutoffs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Afla B (B1 and B2)</td>
<td>1–50 (Fluorometer) 0.1–50 ppb (HPLC)</td>
<td></td>
</tr>
<tr>
<td>AflaCheck</td>
<td>10 and 20 ppb cutoffs</td>
<td></td>
</tr>
<tr>
<td>AflaM, FL+</td>
<td>12.5–200 ppt</td>
<td></td>
</tr>
<tr>
<td>AflaM, HPLC</td>
<td>0.01–3 ppb</td>
<td></td>
</tr>
<tr>
<td>AflaTest</td>
<td>0.1–300 ppb (Fluorometer) 0.05–100 ppb (HPLC)</td>
<td></td>
</tr>
<tr>
<td>AflaTest WB</td>
<td>0.03 (B1) 0.03–400 ppb</td>
<td></td>
</tr>
<tr>
<td>AflaTest WB SR</td>
<td>0.005–500 ppb</td>
<td></td>
</tr>
<tr>
<td>AflaOchr HPLC</td>
<td>0.25–100 ppb (aflatoxins)</td>
<td></td>
</tr>
<tr>
<td>AOX HPLC</td>
<td>0.1–100 ppb (aflatoxins)</td>
<td></td>
</tr>
<tr>
<td>AflaV</td>
<td>2–100 ppb</td>
<td></td>
</tr>
</tbody>
</table>

Get Test Kit Info

Email VICAM
Background (cont.)

Useful Links...

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**AflaTest**

AflaTest is a quantitative method for the detection of aflatoxin in many commodities. VICAM's advanced biotechnology permits the measurement of aflatoxins (including APB1, APB2, AF-G1, AF-G2 and AFM1). The AflaTest mycotoxin testing system can be used in a wide variety of locations from the local farm elevator to food processing quality control laboratories to government testing laboratories.

- **Datasheet**: AflaTest (912 KB)
- **Manual**: AflaTest Fluorometer (914 KB)

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**Benefits**

- **Convenient** — For use with fluorometer or HPLC
- **Comprehensive** — Total readings for all aflatoxins
- **Durable** — Long shelf life, requires no refrigeration
- **Versatile** — For use with a variety of samples
- **Quick** — Less than 10 minutes to isolate toxin
- **Wide Range** — Detects levels as high as 300 ppp and as low as 0.1 ppp

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**Demo Videos**

- **VICAM Afla FGIS Com**: AflaTest Procedure
- **Afla FGIS Com**: AflaTest Procedure

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**AFLATOXIN TESTING SOLUTIONS**

- Afla B
- AflaChack
- Afla M1 FL+
- Afla M1 HPLC
- Afla M1+V
- AflaOctox HPLC
- AflaTest
- AflaTest WB
- AflaTest WB SR
**U.S. federal government action level for aflatoxin**

<table>
<thead>
<tr>
<th>Product</th>
<th>Total aflatoxins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food for human consumption and dairy feed</td>
<td>20ppb</td>
</tr>
<tr>
<td>Milk for human consumption</td>
<td>0.5 ppb</td>
</tr>
<tr>
<td>Nondairy animal feeds</td>
<td>Levels up to 300 ppb</td>
</tr>
</tbody>
</table>
Background (cont.)

Peanut industry level/many food manufacturers

<table>
<thead>
<tr>
<th>Product</th>
<th>Total aflatoxins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peanuts</td>
<td>10ppb</td>
</tr>
</tbody>
</table>
### European community commission regulation

<table>
<thead>
<tr>
<th>Products</th>
<th>B1 (µg/kg)</th>
<th>B1+B2+G1 +G2 (µg/kg)</th>
<th>1 (µg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groundnuts and dried fruit for human consumption</td>
<td>2</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Cereals for human consumption</td>
<td>2</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Milk</td>
<td></td>
<td></td>
<td>0.05</td>
</tr>
</tbody>
</table>
• **Maximum tolerance values**
  
  
  - **2 µg/kg** *(AFB1)* (EFSA, 2007. The ESFA J. 446: 1-127)
  
  - **8 µg/kg** in maize (CODEX Alimentarius, 2006)
  
  
  
Sampling
Background (1)

- **Sampling for aflatoxin analysis**
  - Representative sample deserves particular consideration since a wrong sampling plan can greatly affect the reliability of the measured levels of mycotoxins (aflatoxin) (Whitaker, 2003).
  - Sampling is one of the most important steps that contribute to the variability of analyses due to the non-homogeneous nature of aflatoxins distribution in foods and feed (FAO, 2004; Miraglia et al., 2005). **Hot spots**
  - Skewed distribution (most samples are below a mean of many samples in the lot)
Sampling: Background (2)

Actual contamination 3/72; 0.042 units

- Uncontaminated product
- Contaminated product *(assume same level)*

<table>
<thead>
<tr>
<th>Samples</th>
<th>levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>12x</td>
<td>0.0</td>
</tr>
<tr>
<td>4x</td>
<td>0.125</td>
</tr>
<tr>
<td>1x</td>
<td>0.22</td>
</tr>
<tr>
<td>Mean</td>
<td>0.042</td>
</tr>
</tbody>
</table>
Sampling: Background (3)

- **Distribution of aflatoxin within a batch of peanuts** (Coker et al., 1995: Nat. Toxins 3, 257).

<table>
<thead>
<tr>
<th>Commodities</th>
<th>No samples</th>
<th>Sample size</th>
<th>Range , ppb</th>
<th>Mean, ppb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peanut kernels</td>
<td>84</td>
<td>100</td>
<td>0-4235</td>
<td>209.1</td>
</tr>
<tr>
<td>Peanut cake</td>
<td>204</td>
<td>100</td>
<td>87-249</td>
<td>163.0</td>
</tr>
<tr>
<td>Peanut meal</td>
<td>130</td>
<td>100</td>
<td>40-265</td>
<td>105.5</td>
</tr>
</tbody>
</table>


<table>
<thead>
<tr>
<th>Commodities</th>
<th>No samples</th>
<th>Sample size</th>
<th>Range , ppb</th>
<th>Mean , ppb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single batch of peanut butter</td>
<td>77 jars</td>
<td>300</td>
<td>&lt; 5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>tested</td>
<td></td>
<td>5-10</td>
<td></td>
</tr>
</tbody>
</table>
Sampling methods (2)

• **Pest infestation**
  – Grain which is heavily infested with insect pests should not be sampled

• **Record-keeping**
  – Records must be kept of all sample data
  – Determine the GPS coordinates of each sample location
Sampling methods (3)

• **Storage of samples**
  – Samples should be properly stored overnight in cardboard boxes
  – ‘plastic bags’ should be avoided

• **Labelling of samples**
  – properly labelled on outside of bag (and inside with a piece of paper) with the correct sample number and country code

• **Transportation of samples**
  – Samples should be transported to reference laboratory as soon as possible and kept free of insect infestation, rodent damage or moisture damage (e.g. rain, humidity) in transit and in the reference laboratory.
Pipetting techniques
Accurate pipetting techniques (1)

Types of pipette

- Monochannel
- Stepper
- Multichannel
- Multi-stepper

For fixed and adjustable volume
Accurate pipetting techniques (2)

- **Air displacement**
  - Air between liquid and piston
  - Length and diameter of piston define amount
  - Watery liquids
  - Sensitive for contamination
  - Lower accuracy
  - Cheap

- **Positive displacement**
  - Contact between liquid and piston
  - Length, diameter and tip of piston makes amount
  - Viscosity and volatile liquids
  - Non-sensitive for contamination
  - Good accuracy
  - Expensive

10/1/2015
Accurate pipetting techniques (3)

- **Forward**
  - Only for watery liquids

- **Reverse (Best technique)**
  - Best for viscosity and volatile liquids
  - Best for small volumes
  - Only with air displacement pipet
Accurate pipetting techniques (4)

• How to pipet correctly?
  – Choice
  – 50-200 μl
  – 100-300 μl
  – 200-500 μl
  – Rinse tip
  – First stop (not in liquid)
  – Fill tip (wait 1 sec)
  – Clean tip
  – Empty tip
    30-70°
    Not in liquid
    1 sec between first and second stop
    Do not rinse

Never use a pipet outside its range!!!
Accurate pipetting techniques (5)

• **Volume ~**
  - Vertical movement
  - Diameter

• **Incorrect volume**
  - Change diameter
    - No maintenance
    - Liquids in pipet
      - Open, clean, dry and calibrate
  - Liquid colder/warmer than pipet
  - $\neq 0,6 \mu l$ per °C
  - Tips
    - By hand = rinse 5x
    - Out of box = rinse 2x
Accuracy and Precision (6)

- Accurate and precise
- Not accurate and precise
- Accurate and Not precise
- Not accurate and Not precise

Maintenance and repair

- Adjust
- Ok
Tips & Tricks (7)

• Inside of tip holder accidentally gets wet
  – Do NOT continue pipetting
    • **Open pipet, clean & calibrate**

• Store pipettes in an upright position

• **Volume adjustment** cfr. Gilson pipettes (e.g. 10; 22; 20)

• **Volatile liquids**
  • 1) Positive displacement pipet
  • 2) Stepper
  • 3) Air displacement pipet + rinsing (3 times)

10/1/2015
Tips & Tricks (8)

• **Brand** of the pipet tips
  • Best results with tip of the same brand
  • In case of different brand
    – PLT-unit / manual check

• **Detergents**: Use of Ultra **Low retention** tips
Testing methodology
Overview of analytical methods

- Enzyme-linked immunosorbent assay (ELISA),
- Lateral flow devices (LFD)
- Liquid chromatographic (LC),
- Immunoaffinity column (aflatest, fumonitest, etc.),
- Multifunctional column,
- Gas chromatography (GC),
- Thin layer chromatography (TLC)
- High performance liquid chromatography (HPLC and UPLC)
- Near Infrared spectroscopy (NIR)
- Electronic nose (e-nose)

Enzyme-linked immunosorbent assay (ELISA)

- Specific to each aflatoxin analogue,
- Most of the time specific to AFB1 (crops) or AFM1 (milk)
- No clean up
- Matrix interference
- Low sensitivity
- Longer time for the assay (~75 min.)
Thin Layer Chromatography

• Each aflatoxin analogue and total,
• No clean up
• High sensitivity
• longer time for the assay (~30 min.)
Lateral flow devices

- Specific to each aflatoxin analogue,
- Most of the time specific to AFB1 (crops) or AFM1 (milk)
- No clean up
- Matrix interference
- Low sensitivity
- Short time for the assay (~15 min.)