What are the Health Effects of Aflatoxin?

Population Perspective

John D. Groopman

Why Aflatoxin?

• Paradigm for complex problems in environmental health
• Naturally occurring carcinogen produced by fungal contamination of grains
  – If it was synthetic, then it would be banned under the 1958 Delaney Amendment
  – Regulatory levels have been established for foods
• Levels affect the price of commodities in international trade
• Human carcinogen contributing in part to over 850,000 deaths per year
• Major problem for growth and development in both animals and humans
• Primary and secondary prevention strategies have been established
Outline

Population Perspective

• Aflatoxin: What is it? Where does it come from?

• Etiology of Human Liver Cancer: Environmental Exposures and Disease

Molecular Perspective

• Strategies for Prevention of Liver Cancer: Application of Biomarkers
Aflatoxin factoids

- Discovered in UK ~1960 in moldy, toxic animal feed

**TURKEY "X" DISEASE**

By W. P. Blount,
T.D., Ph.D., F.R.C.V.S., F.P.H., F.R.S.E.

(Director and chief Poultry Adviser, BOOM Ltd)

**CONFERENCES PAPERS**

The first real suspicion we had that anything might be amiss with turkeys last occurred in May when there were several instances by well-known farmers to the effect that their birds were not finding their usual appetite, and were not eating as usual. A majority of proprietary feeds were concerned, and no straightforward answer to this question was forthcoming at the time.

In June there were reports from the field that several flocks were dying in large numbers and no typical disease features were in evidence. Post-mortem examinations showed that organs were common, but it was not very specific, sometimes being extraneous and at other times haemorrhagic. There was no unusual gastrointestinal appearance that suggested to me that some form of poisoning had taken place. But what? The birds were nearly all sick and lifeless, many being unable to move, and within half an hour more than a dozen others had died. Here indeed was the problem!

**Symptoms**

Many affected turkeys were about 4-6 weeks old, but others were aged 15-16 weeks. Actually it is very doubtful whether any poultry younger than two weeks of age or older than 18 months by 15-20 weeks, or breeding stock, dying from this malady.
WHICH TURKEY WAS FED AFLATOXIN?

(hint)

Aflatoxins in Human Food

Corn
Wheat
Rice
Peanuts
Soybeans

Oats
Sorghum
Millet
Cottonseed
Copra
To all whom it may concern:  

Be it known that I, JOHN H. KELLOGG, of Battle Creek, in the county of Calhoun and State of Michigan, have invented a new and useful Process of Manufacturing an Improved Alimentary Nut Product, of which the following is a specification.

It is the object of my invention to produce an improved alimentary product or products from peanuts or other nuts. To this end I subject the nut-kernels to the process hereinafter described, whereby I obtain a bifold or double product, namely, a dry and practically white nutmeal and a pasty adhesive substance that is for convenience of distinction termed "nut-butter."
PEANUT BUTTER FACTOIDS

• Peanut butter will celebrate its 120th birthday as a sandwich spread in 2015
• It is consumed daily by approximately 40 million Americans
• Americans prefer creamy peanut butter to the crunchy variety 60% to 40%
• Elvis Presley’s favorite sandwich was grilled peanut butter with bananas
• The average child will eat 1,500 peanut butter sandwiches by the time he or she graduates from high school
• 85% of Americans have a jar of peanut butter in their homes
• Each year Americans consume enough peanut butter to coat the entire floor of the Grand Canyon

Source: USA Today, adapted
### U.S. Food and Drug Administration and European Union Guidelines for Acceptable Levels of Aflatoxins in Food and Feed

<table>
<thead>
<tr>
<th>Action level (ppb)</th>
<th>Commodity</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 (aflatoxin M₁)*</td>
<td>milk</td>
<td>humans</td>
</tr>
<tr>
<td>5 - 20.0</td>
<td>any food (peanuts) except milk</td>
<td>humans</td>
</tr>
<tr>
<td>5 - 20.0</td>
<td>feed</td>
<td>all species</td>
</tr>
</tbody>
</table>

#### Exceptions

<table>
<thead>
<tr>
<th>Action level (ppb)</th>
<th>Commodity</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 - 300.0</td>
<td>cottonseed meal used in feed</td>
<td>all species</td>
</tr>
<tr>
<td>50 - 300.0</td>
<td>corn</td>
<td>finishing beef cattle</td>
</tr>
<tr>
<td>50 - 200.0</td>
<td>corn</td>
<td>finishing swine</td>
</tr>
<tr>
<td>(&gt;100#)</td>
<td>corn</td>
<td>breeding cattle, swine, and poultry</td>
</tr>
<tr>
<td>100.0</td>
<td>corn</td>
<td></td>
</tr>
</tbody>
</table>

* Specifically for aflatoxin M₁, a toxic metabolite of AFB₁ that occurs in milk and processed to cheese (10 fold concentration)

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**Dr. M.M. Abdel Kader views Thin Layer Chromatography (TLC) of aflatoxins in UV light: 1963**

The action level was established based upon what could be visibly seen by TLC chromatography
Top exporters of maize worldwide
(threshold = 1 million MT)

Sampling and testing:
Is 20 ppb an appropriate regulation for aflatoxin contamination?
Coefficient of Variation In Analysis of Aflatoxin In a Heterogeneous Lot: The Regulatory Action level is 20 ppb

C.V. is 100% or 20 ± 20 ppb

C.V. is 15% or 20 ± 3 ppb

10 pound sample

1 pound sub-sample for analysis

INDIVIDUAL PEANUTS WERE MEASURED


The Toxicological Paradigm

exposure

internal dose

biologic effective dose

early biologic effects

altered structure & function

clinical disease

Susceptibility genetic factors

Effect modifiers diet habits health medication co-exposure
Outline

Population Perspective

- Aflatoxin: What is it? Where does it come from?
- Etiology of Human Liver Cancer: Environmental Exposures and Disease

Molecular Perspective

- Strategies for Prevention of Liver Cancer: Application of Biomarkers

Liver Cancer Mortality (2018)

- Liver cancer is a leading cause of global cancer death; most before age 50; 8.2% of total (2018)
- >80% of HCC occurs in the developing world
- ~500 million HBV carriers worldwide
- ~160 million HCV infected people worldwide
- ~100 million liver cancer deaths in 21st century
- etiology of 90-95% of liver cancer now known

826,000 deaths in 2016 (WHO)
Baltimore population 619,362
Etiology of Human Liver Cancer

- HBV
- HCV
- alcohol
- emerging factors: NAFLD, T2D, NASH
- aflatoxins
**Percent of cancer attributable to alcohol, 2010**

<table>
<thead>
<tr>
<th>Cancer Type</th>
<th>Total</th>
<th>Women</th>
<th>Men</th>
<th>Percentage of Deaths Attributable to Alcohol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouth cancer</td>
<td>26.8%</td>
<td>29.3%</td>
<td>24.9%</td>
<td>20.8%</td>
</tr>
<tr>
<td>Cancer of the nasopharynx</td>
<td>27.4%</td>
<td>27.9%</td>
<td>27.0%</td>
<td>5.6%</td>
</tr>
<tr>
<td>Cancer of other parts of pharynx and larynx</td>
<td>20.8%</td>
<td>5.6%</td>
<td>20.8%</td>
<td>13.2%</td>
</tr>
<tr>
<td>Oesophageal cancer</td>
<td>5.6%</td>
<td>5.5%</td>
<td>5.7%</td>
<td>5.8%</td>
</tr>
<tr>
<td>Cancers of the colon and rectum</td>
<td>13.2%</td>
<td>13.5%</td>
<td>13.2%</td>
<td>8.0%</td>
</tr>
<tr>
<td>Laryngeal cancer</td>
<td>22.5%</td>
<td>22.5%</td>
<td>22.5%</td>
<td>9.0%</td>
</tr>
<tr>
<td>Cancer of the female breast</td>
<td>9.0%</td>
<td>9.0%</td>
<td>9.0%</td>
<td>9.0%</td>
</tr>
<tr>
<td>All cancer deaths due to alcohol</td>
<td>6.0%</td>
<td>6.0%</td>
<td>6.0%</td>
<td>6.0%</td>
</tr>
</tbody>
</table>

**World Cancer Report, 2014**

**Global Burden of Diabetes**

- **International Diabetes Foundation Atlas 5th Edition 2012**

Burden of HCC Attributable to Aflatoxin: 25,200 – 155,000 cases/year
### Aflatoxin and Acute Disease

Table III. Aflatoxicosis in maize-consuming communities

<table>
<thead>
<tr>
<th>Population</th>
<th>Fatalities</th>
<th>Samples</th>
<th>Estimated intake$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>India (1974): 307 patients in &gt;180 villages in western India</td>
<td>106 died (27% fatality)</td>
<td>Maize from affected household contained aflatoxin (type unspecified) levels between 6250 and 15 600 p.p.b.</td>
<td>Intakes: 6.25–15.6 p.p.m. aflatoxins and 350 g maize/day equals to 2.19–5.46 mg aflatoxins; 36.5–91 μg aflatoxin/kg/day.</td>
</tr>
<tr>
<td>Kenya (1981): 20 cases in Machakos district</td>
<td>12 died (60% fatality)</td>
<td>Maize from homes with fatalities had 2,000 and 12,000 p.p.b. of AFBI; 500 p.p.b. was not associated with fatality; two necropsy liver samples contained 39 and 89 p.p.b. of AFBI</td>
<td>Intakes: 3.2–12 p.p.m. AFBI and 350 g maize/day equals to 1.12–4.2 mg AFBI; 18.7–70 μg AFBI/kg/day.</td>
</tr>
<tr>
<td>Kenya (2004): 317 cases in Eastern Kenya (Makueni, Kitui, Machakos and Thika). Case-control study of 40 cases with acute jaundice and 80 village controls</td>
<td>125 deaths (39% fatality). In the case-control study, 29 cases alive at the time of blood sampling, an additional seven died by August 2004. GM of total aflatoxins in stored household maize*: 354.53 p.p.b. in case and 44.14 p.p.b. in control households. Median AFBI-tyrosine 0.25 ng/ml; in cases 0.25 ng/ml and in controls 0.15 ng/ml; fatality rates higher addicts than survivors among cases: GM 3.2 versus 0.5 ng/ml</td>
<td>Intakes: 5–20 p.p.m. were associated with fatality and 350 g maize/day equals to 1.75–7 mg aflatoxins; 29.2–116.7 μg/kg/day total aflatoxin,</td>
<td></td>
</tr>
</tbody>
</table>

Aflatoxin Causes Deaths in People
**Imputed Human LD_{50}**

<table>
<thead>
<tr>
<th>Species</th>
<th>Dose (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit</td>
<td>0.3</td>
</tr>
<tr>
<td>Cat</td>
<td>0.6</td>
</tr>
<tr>
<td>Dog</td>
<td>0.5–1.0</td>
</tr>
<tr>
<td>Pig</td>
<td>0.6</td>
</tr>
<tr>
<td>Baboon</td>
<td>2.0</td>
</tr>
<tr>
<td>Rat (male)</td>
<td>5.5</td>
</tr>
<tr>
<td>Macaque monkey</td>
<td>7.8</td>
</tr>
<tr>
<td>Mouse</td>
<td>9.0</td>
</tr>
<tr>
<td>Hamster</td>
<td>10.2</td>
</tr>
<tr>
<td><strong>Human</strong></td>
<td><strong>0.54–1.62</strong></td>
</tr>
</tbody>
</table>

Aflatoxin and Child Health

Stunting in children

• Definition
  – Child’s height for his/her age is 2 standard deviations or more below WHO growth reference (HAZ ≤ -2)
  – Associated with cognitive impairment & increased vulnerability to infectious disease (Ricci et al. 2006)

• Prevalence
  – 195 million stunted children under age 5 worldwide (Black et al. 2008)
Exposure to Aflatoxin Associated with Reduced Growth in West African Children

BMJ. (2002) 325:20-1
# Studies linking aflatoxin to growth impairment in children

<table>
<thead>
<tr>
<th>Type of study</th>
<th>Results</th>
<th>Nation &amp; study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aflatoxin measurements in stored flour, rural homes</td>
<td>Stunting, underweight, &amp; wasting associated with higher AF levels in flour</td>
<td>Kenya (Okoth &amp; Ohingo 2004)</td>
</tr>
<tr>
<td>Cross-sectional: AF-alb levels in maternal, cord, child blood</td>
<td>Stunting &amp; underweight associated with higher AF-alb levels in these fluids</td>
<td>Togo, Benin, United Arab Emirates, The Gambia (Gong et al. 2002*, Abdulrazzaq et al. 2004, Turner et al. 2007)</td>
</tr>
<tr>
<td>Longitudinal: AF-alb levels in children’s blood</td>
<td>Reduced height gain in 8 mos associated with AF-alb levels</td>
<td>Benin (Gong et al. 2004)</td>
</tr>
<tr>
<td>AFM1 in mothers’ breastmilk</td>
<td>Lower length at birth &amp; in infancy associated with AFM1</td>
<td>Iran (Sadeghi et al. 2009, Mahdavi &amp; Nikhniaz 2010)</td>
</tr>
</tbody>
</table>


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# Aflatoxin and Chronic Disease: HCC
Types of Observational Studies to Assess Risk

- Cross-sectional studies (surveys)
  - Observations made on exposure and disease at a single point in time
- Case control studies (retrospective)
  - Individuals with a given disease (cases) are compared with persons without the disease
- Cohort studies (prospective)
  - In a cohort study the investigator selects a group of exposed and a group of non-exposed individuals and follows the two groups to compare the incidence of disease

Observational epidemiology provided early evidence that aflatoxin is a risk factor for HCC

- Frequent contaminant of human diets in developing countries
- Estimated intake correlates positively with HCC incidence
**Incidence of liver cancer in men is associated with aflatoxin ingestion**

Aflatoxin intake quantified by analysis of food as eaten, HCC incidence from registry data


![Graph showing the relationship between aflatoxin intake and liver cancer incidence.](Bosch and Munoz. IARC Publ. No. 89: 427 (1988) Modified)

**HBV surface antigen in serum identifies men at high risk**

Prospective study in 22,707 Chinese men in Taiwan

<table>
<thead>
<tr>
<th>HBsAg Status</th>
<th>Relative Risk for Liver Cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBV Antigen Negative</td>
<td>1.0</td>
</tr>
<tr>
<td>HBV Antigen Positive</td>
<td>98.4</td>
</tr>
</tbody>
</table>

- Demonstrates the power of an immunologic biomarker
- However, variation in HCC rates could not be explained by this one metric alone.

*Beasley et al., The Lancet 2: 1129, (1981)*
Geographic variation of HCC incidence in male HBV carriers suggests environmental risk factors

T. London (1994)

LIVER CANCER (MALE)

• 2nd leading cause of cancer death in China
• 300,000 deaths per year
• <10% 1 year survival
The Toxicological Paradigm

exposure

internal dose

biologic effective dose

early biologic effects

altered structure & function

clinical disease

Susceptibility genetic factors

Effect modifiers diet habits health medication co-exposure

Aflatoxin-N7-guanine (urine)

Aflatoxin-albumin adduct (serum)

Aflatoxin-Mercapturic Acid (urine)
Aflatoxin DNA Damage Products Excreted into Urine: Biologically Effective Dose Biomarker

![Graph showing the relationship between total aflatoxin B1 intake and total aflatoxin-N7-guanine in urine with a correlation coefficient of r=0.80.](image)

Groopman et al Cancer Research 52:45-52, 1992

Cohort Study of Liver Cancer in P.R.C.: Viral-Chemical Interactions

- 18,244 urine and blood samples collected from healthy men age 45-65
- 50 liver cancer cases and 247 controls
- Urinary aflatoxin biomarkers measured in blinded samples
- HBV status determined for each subject

<table>
<thead>
<tr>
<th>BIOMARKERS: HBsAg AND URINARY AFLATOXINS</th>
<th>RELATIVE RISK FOR LIVER CANCER</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO BIOMARKERS DETECTED</td>
<td>1.0</td>
</tr>
<tr>
<td>HBV (YES) AFLATOXIN (NO)</td>
<td>7.3</td>
</tr>
<tr>
<td>HBV (NO) AFLATOXIN (YES)</td>
<td>3.4</td>
</tr>
<tr>
<td>HBV (YES) AFLATOXIN (YES)</td>
<td>60.0</td>
</tr>
</tbody>
</table>

The Toxicological Paradigm

exposure

internal dose

biologic effective dose

early biologic effects

altered structure & function

clinical disease

Susceptibility
genetic factors

Effect modifiers
diet
habits
health medication
co-exposure

Mortality from Liver Cancer by Township: Jiangsu Province

< 1 per 10^5/yr

> 50 per 10^5/yr

25-fold change in HCC rate in 200 km

- Median age of liver cancer death is 45-50 years
- Median survival from Dx is 6 months
• codon 249 p53 mutations are detectable in DNA in human plasma samples
• strong mechanistic studies link aflatoxin with this p53 mutation in codon 249
• **LIQUID BIOPSY**: detect p53 DNA 5 years prior to Dx
Genetic and molecular evidence that AFB₁ is a human carcinogen

- **p53, the most commonly mutated gene in human cancer, has a unique mutation in liver tumors**
  - Major mutation in p53 at codon 249 G:C to T:A mutation is related to aflatoxin exposure
  - May represent “early effect” genetic biomarker

- **Mutations induced by aflatoxin**
  - G:C to T:A is the predominant mutation induced by AFB₁ in bacteria, plasmids and human gene assays
  - p53 codon 249 mutation is preferentially induced in human liver cells by aflatoxin

- **Molecular mechanism of aflatoxin-DNA binding**

Aflatoxin fulfills Hill criteria for causation vs association

- Strength of association
- Consistency
- Specificity
- Temporality
- Biological gradient
- Plausibility
- Coherence
- Experiment

On the basis of the totality of evidence, IARC classified aflatoxin as Group 1 known human carcinogen in 1993.

What are the Health Effects of Aflatoxin?

Molecular Perspective

John D. Groopman
# Outline

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## Strategies for Aflatoxin Prevention

**Primary**

- Reduced aflatoxin consumption:
  - improve food storage
  - biocontrol
  - changes in dietary staples

**Secondary**

- Chemopreventive interventions: e.g., oltipraz, broccoli sprouts, chlorophyllin, green tea
Strategies for Aflatoxin Prevention

- Reduced aflatoxin consumption:
  - improve food storage
  - biocontrol
  - changes in dietary staples

Intervention Package in Kindia, Guinea

Intervention villages

- **Hand sorting:** Training in removal of groundnuts that were visibly mouldy or had the shells damaged
- **Drying on mats:** Provision of locally produced natural fibre mats for the sun drying process
- **Sun drying:** Training in sun drying by shaking the kernels to listen for the free movement of the dried nuts
- **Storage in natural fibre bags:** Provision of natural fibre jute bags to replace plastic or other synthetic bags
- **Wooden pallets:** Provision of locally made wooden pallets on which to store the bags
- **Insecticide:** Provision of locally available insecticide (acetilite) to sprinkle on the floor of the storage facility

Control villages: farmers were left to follow their normal post-harvest practices

Levels of Aflatoxin Biomarkers are Reduced in Children in West Africa by Primary Intervention

Trial Samples


Strategies for Aflatoxin Prevention

- Chemopreventive interventions: e.g., oltipraz, broccoli sprouts, chlorophyllin, green tea
CANCER CHEMOPREVENTION

- Retardation, blockage or reversal of carcinogenesis (before malignancy) by synthetic or natural agents

(e.g., drugs (Tamoxifen), foods, supplements)

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**Quantitative Rat Model: Tracking Biomarkers**

- Oltipraz is 100% effective for chemoprotection against AFB$_1$ induced HCC in rats

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Key Steps in Translation

- Demonstrate protective efficacy in animals
- Demonstrate bioavailability and pharmacodynamic action in target tissue in animal model
- Establish kinetic and dynamic correlates in humans
- Efficacy in humans

Chemoprevention Studies in Hepatocellular Carcinoma

- Median age of liver cancer death is 45-50 years
- Median survival from Dx is 6 months
- Five clinical trials, to date, using biomarkers

25-fold change in HCC rate in 200 km

- **Oltipraz**: increased aflatoxin-mercapturic acid excretion in urine (Wang et al., JNCI, 1999)
- **Chlorophyllin**: decreased aflatoxin-DNA adduct excretion in urine (Egner et al., PNAS, 2001)
- **Sulforaphane**: decreased aflatoxin-DNA adduct excretion in urine (Kensler et al, CEBP, 2005)
- **Broccoli sprout extract**: modulates metabolism (Egner et al, Cancer Prevention Research, 2011).
- **Glucoraphnin/Sulforaphane**: dose escalation and pill formulation (July 2015, January 2016, January 2018)
* In F344 rat model a 55% reduction in DNA adducts results in greater than 97% reduction in liver tumors

Wang et al., *JNCI* 91: 347-353, 1999


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**Induction of Detoxication Enzymes by Extracts from 3 Families of Vegetables**

(Paul Talalay - 1992)

- **Broccoli**
- **Brus Sprouts**
- **Kale**
- **Red Cabbage**
- **Green Onion**
- **Tomato**
- **Green Pepper**
- **Red Pepper**
- **Cauliflower**
- **Green Cabbage**
- **Leek**
- **Asparagus**
GLUCORAPHANIN

SULFORAPHANE

Enzyme Induction Potency of Broccoli of Plant Age

Fahey et al. PNAS 1997
How to make the beverage when it is classified as a “drug”

Good Manufacturing Practice (GMP) for Food

150 participants X 84 days = 12,600 doses each for “drug” and “placebo” arms
Reduced up to 10 fold with increasing levels of sulforaphane from sprouts

Kensler et al. *CEBP* 14: 2605-13, 2005

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**Clinical Chemoprevention Trials in Qidong, PRC: Aflatoxin and Air Pollutants**

- **Oltipraz**: increased aflatoxin-mercapturic acid excretion in urine (Wang et al., JNCI, 1999)
- **Chlorophyllin**: decreased aflatoxin-DNA adduct excretion in urine (Egner et al., PNAS, 2001)
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- **Broccoli sprout extract**: modulates metabolism (Egner et al, Cancer Prevention Research, 2011).
- **Glucoraphninin/Sulforaphane**: dose escalation and pill formulation for air pollutants (July 2015, January 2016, January 2018)
Decreasing Liver Cancer Mortality by 50%: An Aflatoxin Story

Age-Specific Rates (China Population) of Liver Cancer are Dropping in Qidong

China Age-Standardized Rate Liver Cancer Incidence in Qidong

CASR men

CASR women
There is a Dramatic Drop in Age-Specific Mortality Rates from Liver Cancer in the Younger Birth Cohorts (after early 1960s)

What is driving the decline in liver cancer in the younger birth cohorts?

- HBV vaccination?
- Reduced aflatoxin exposures?
- Other factors?
  - drinking water
Most of the decline in liver cancer has occurred in birth cohorts never vaccinated against HBV.

If it's not HBV, what about aflatoxin?

Aflatoxin is a significant contaminant of maize and peanuts, but not rice.
RICE CULTIVATION

maize >> rice
aflatoxin contamination

http://www.china-food-security.org/data/maps/crops/all_h.htm

http://www.china-food-security.org/data/maps/crops/all_h.htm
Aflatoxin Exposures Have Dropped Dramatically over the Past 25 Years (>40-fold)

Chen et al, Canc Prev Res, 2013

Declining Liver Cancer by 50% in Rural China from 1990s to Present

Declining Liver Cancer by 50% in Rural China from 1990s to Present

Average Incident Age
Liver Cancer Incidence
China Age Standardized Rate

Percent of Samples Non-Detectable

Aflatoxin-Albumin Adducts (pg/mg albumen)


HBV Vaccination 2002


Average Incident Age
NAFLD T2D Obesity

Key Points 1

- Development and application of validated, *quantitative* exposure biomarkers have identified major etiological factors in HCC.
- Use of these biomarkers in a *quantitative* experimental chemoprevention model establish the efficacy of a spectrum of agents.
- Validated biomarkers identify high-risk people for screening and preventive interventions in populations at risk for HCC.
Key Points 2

• In studies in Africa and most of Asia over the past 25 years over 95% of human samples have detectable aflatoxin albumin adducts (Wild and Gong, 2010).

• Grounding exposure and efficacy of interventions with aflatoxin biomarkers will allow comparison across different studies and strategies for prevention.

• Biomarker levels under predict the efficacy of experimental interventions. A 50% reduction can have a profound impact on chronic disease outcome (Johnson et al, 2014).

• Early life exposures lead to many more cell division opportunities for genotoxic damage

• Mechanistic understanding of growth still needed with models.