Practical strategy to improve food safety and manage aflatoxicosis during outbreaks

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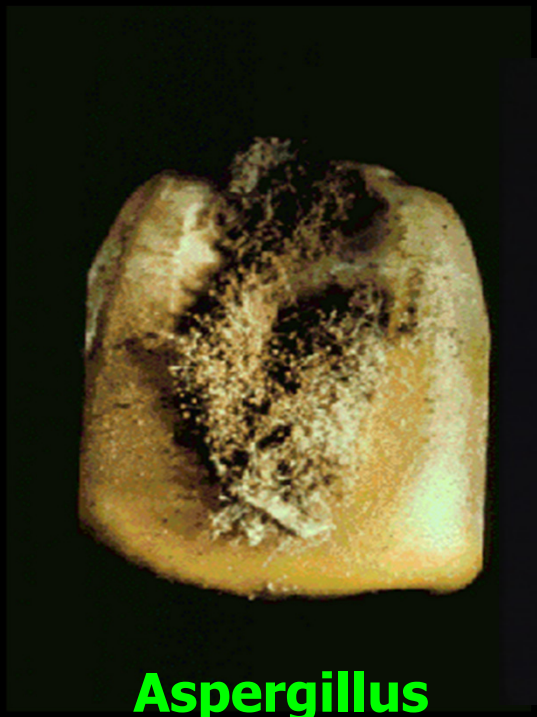
TOXICOLOGY PROGRAM
INTERDISCIPLINARY STUDIES

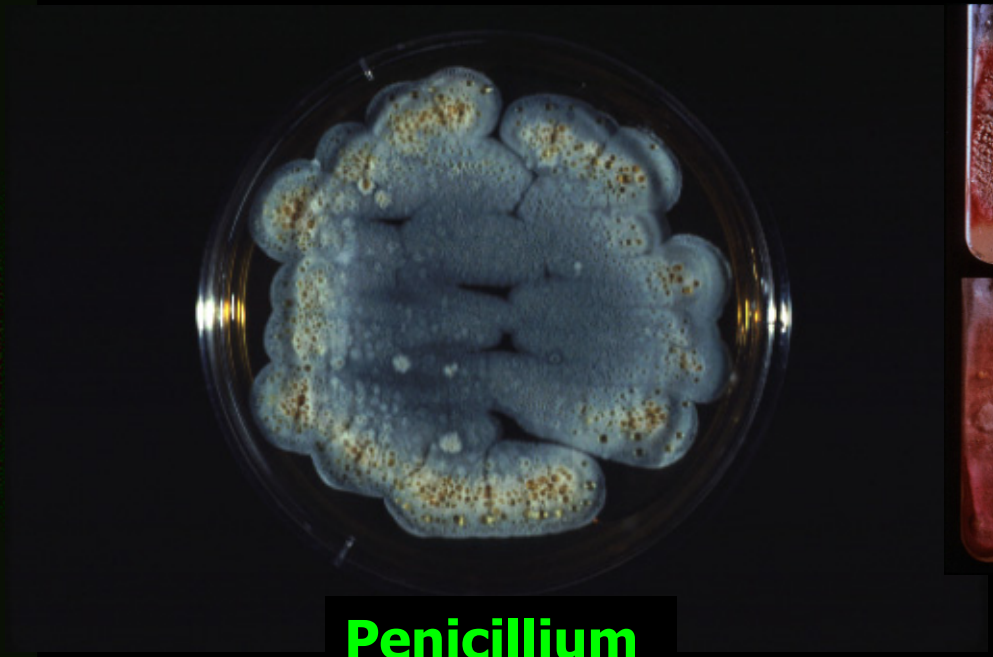
● **Presentation Focus:** **Clay research**

Mycotoxins

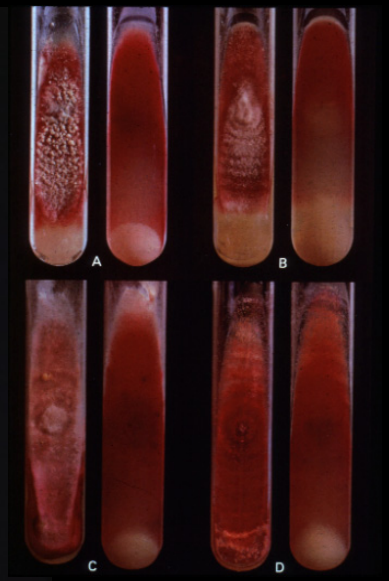
- The problem with mycotoxins is longstanding. Mycotoxins are toxic metabolites of fungi that frequently occur in food and feed and have been strongly implicated in disease and death in humans and animals.



Aspergillus

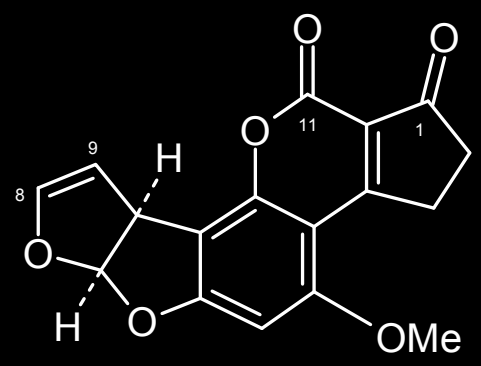


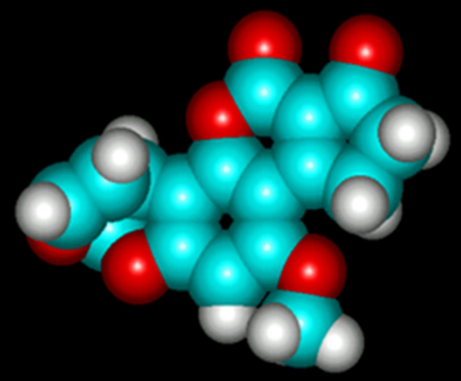
Penicillium

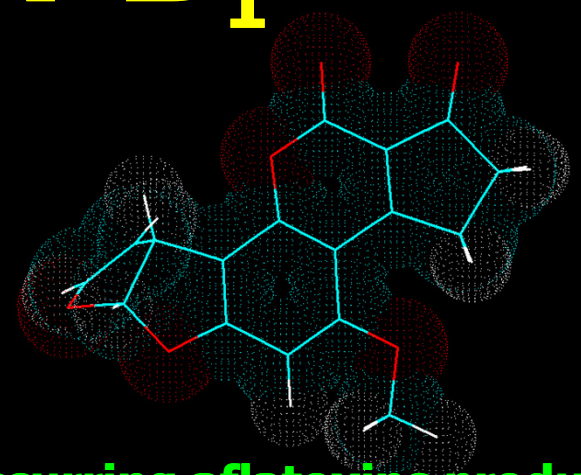


Fusarium

AFLATOXIN B_1







Aflatoxin B_1 is the most potent of 4 naturally-occurring aflatoxins produced by fungi on grains and oilseeds.

- **Aflatoxin B_1 is an important co-factor for hepatocellular carcinoma (HCC). It is also hepatotoxic, immunotoxic, and antinutritional.**
- **Source: *Aspergillus flavus* and *parasiticus* fungi (in the field and in storage).**
- **Drought stress is a common cause of fungal infection and production of aflatoxins. Along with HCC, aflatoxin contamination of food can have an impact on food security, hunger, famine, malnutrition, and infectious disease, especially in children and young animals.**

Aflatoxin Exposure from the Diet

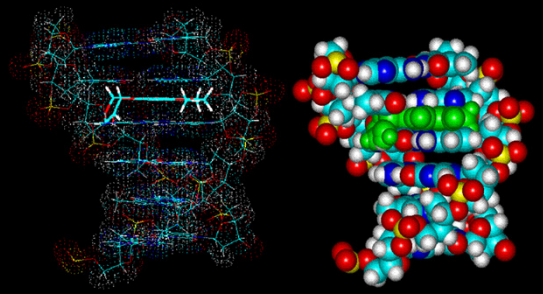
- Is considerably enhanced in developing countries where aflatoxins in the diet of humans and animals are largely uncontrolled (Tropical, semi-tropical zone: Africa, India, China, Latin America and the Carribean, etc.).

- Impacts the vulnerable and the"poorest people" who are most likely to consume foods contaminated with aflatoxins and suffer the consequences. The young are the most susceptible!

The Consequences

- Increased risk of liver cancer from infection with hepatitis.
- Suppression of the immune system. Well-established in animals and likely in humans.
- Significant decreases in serum and liver vitamin A levels and other nutrients in animals (e.g., vitamin D, Se, Zn & Fe).
- Growth retardation & malnutrition are associated with exposure. Most common.
- Acute aflatoxicosis and death.

Hepatocellular carcinoma



AFB1-DNA ADDUCT FORMATION

✓ Examples: India in 1974; Kenya in 2004 with a high case fatality rate (39%) and concentrations in maize up to *8,000 ppb. New cases yearly.

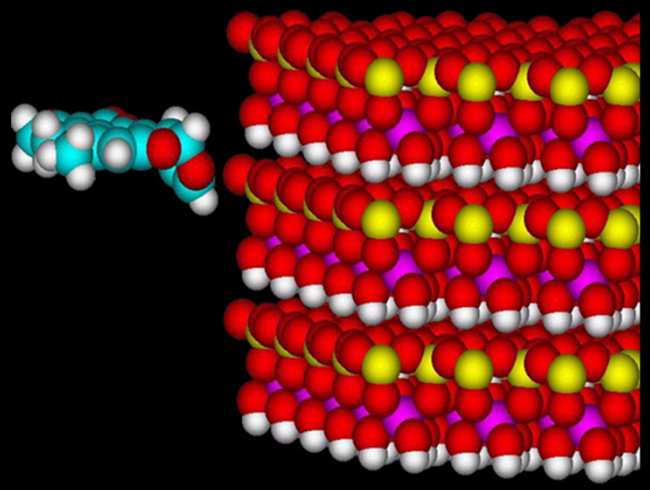
Solving the Problem with Clay

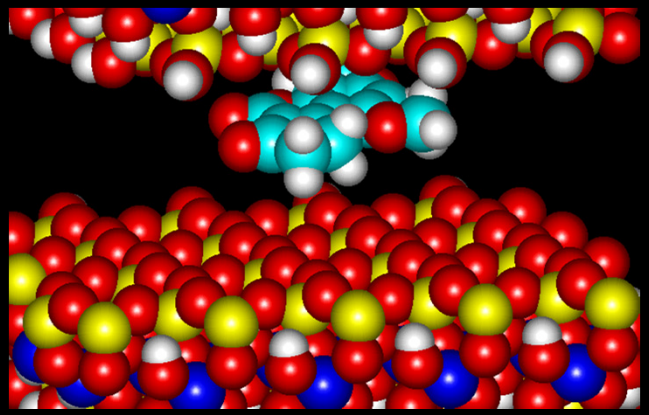
- **HISTORICAL PERSPECTIVE:** Clays have been used as *Ancient Medicine* for diarrhea, wound healing, skin infection and other ailments, as well as binding agents for toxins. The ingestion of clay (geophagy) has been observed for centuries and across all continents. Based on my earlier work, clays are being used worldwide for the treatment of aflatoxin outbreaks in animals, but not humans.

- **MY CURRENT OBJECTIVE:** To develop clay-based therapy, delivered in food and/or water, that can decrease aflatoxin exposures and lethality in people during outbreaks.

Clays commonly consumed by humans and animals

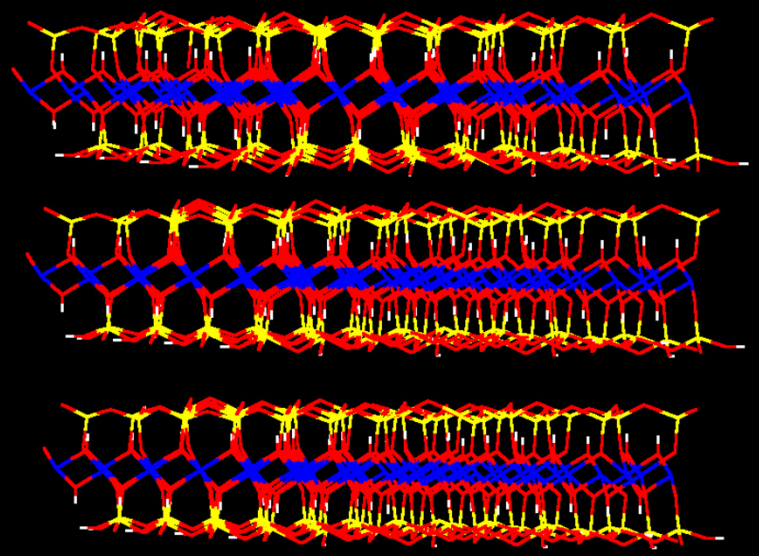
Kaolinites: Commonly eaten due to the soft, chalk-like consistency. Very little interlayer spacing and delamination.





Dioctahedral Smectites: Examples: montmorillonite, nontronite and beidellite clays with sheets of 6-membered rings and variations in framework minerals, interlayer cations and charge distribution. NovaSil™ is a calcium montmorillonite clay.

NovaSil Clay (Binds Aflatoxins)

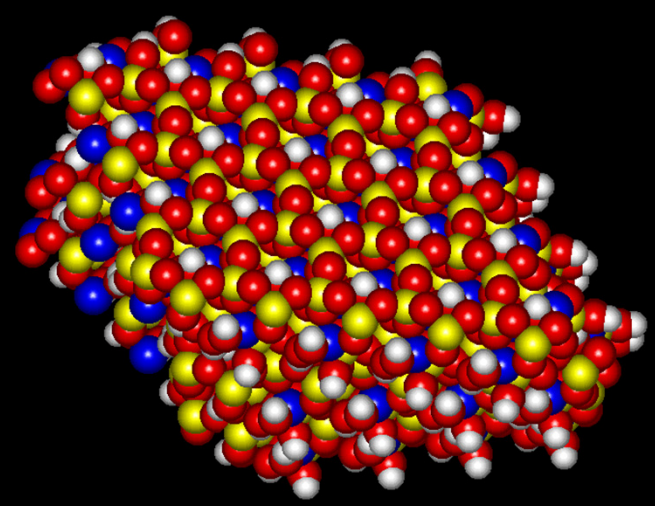


2:1 Layer-lattice aluminosilicate - Negative charge responsible for high cation exchange capacities in soils.

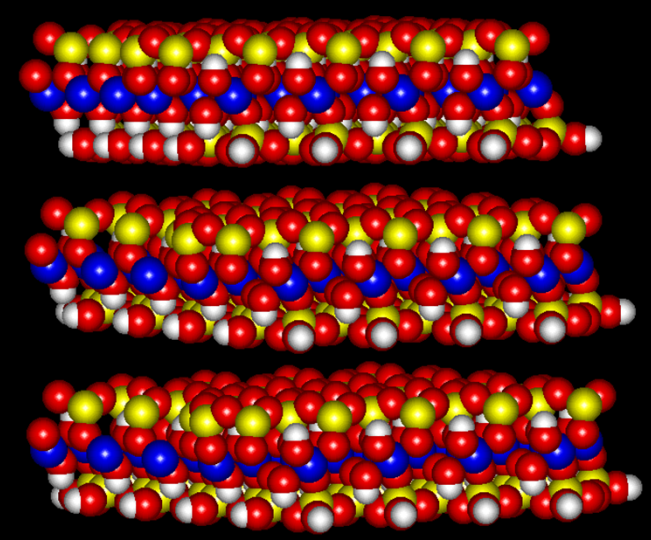
Si^{4+} yellow Silica tetrahedra
Al^{3+} blue Aluminum octahedra

Common substitutions:
Mg^{2+} for Al3+
Al^{3+} for Si^{4+}
Fe^{3+} for Al^{3+}

$(Na, Ca)_{0.3}Al_2Si_4O_{10}(OH)_2 \cdot nH_2O$

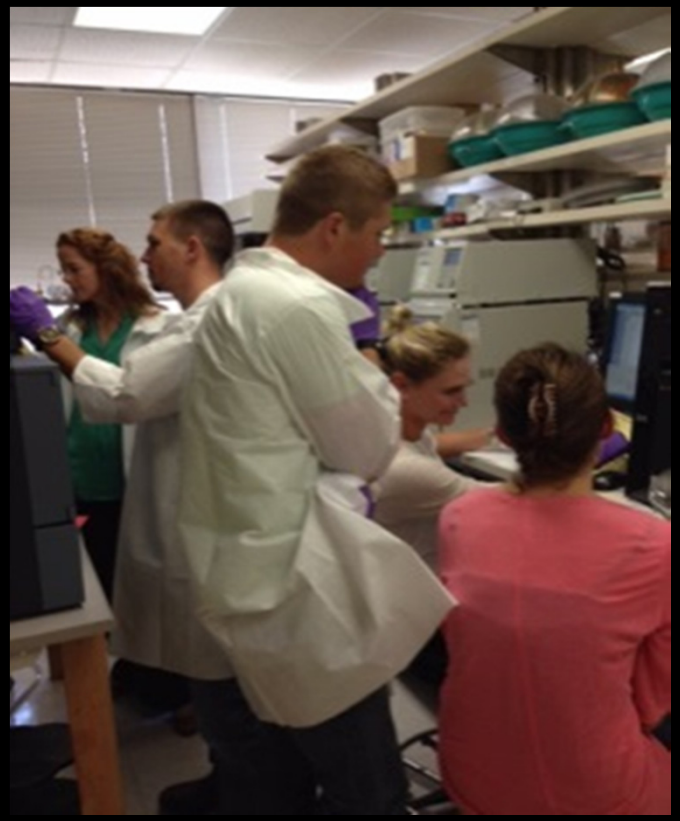


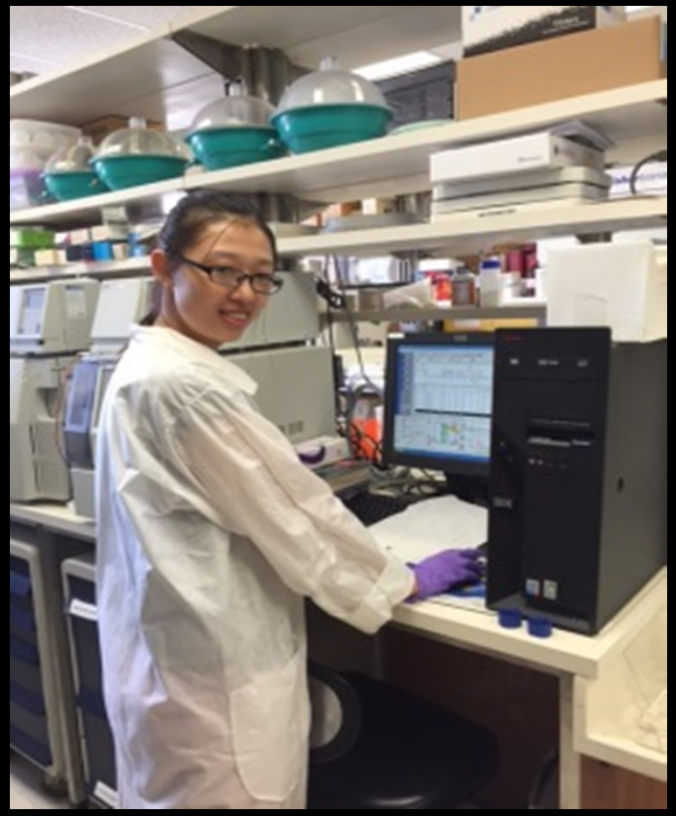
Top view



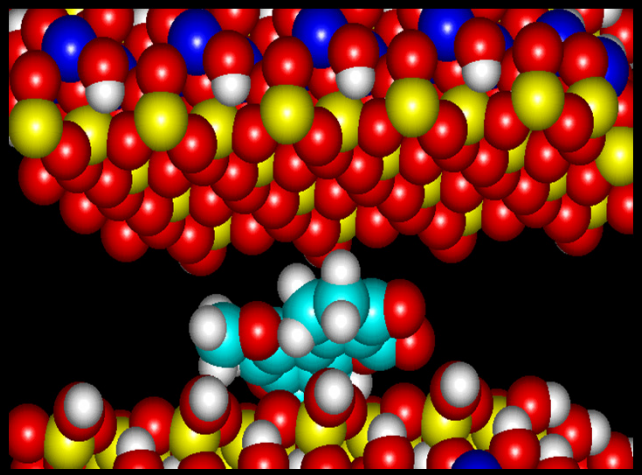
Side view

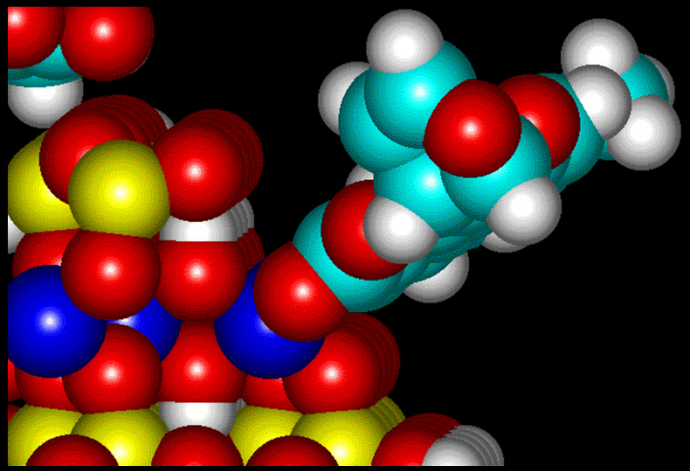
BENCH STUDIES WITH NOVASIL





Evidence for efficacy & specificity of NovaSil clay *in vitro*

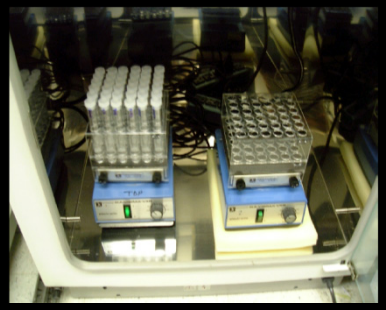




- **Structure/activity** (aflatoxin analogs; binding specificity)
- **Adsorption isotherms** (capacity, affinity, enthalpy)
- **Modeling and Computational Quantum Chemistry** (toxin binding sites and reaction mechanisms)

Methods of isothermal analysis

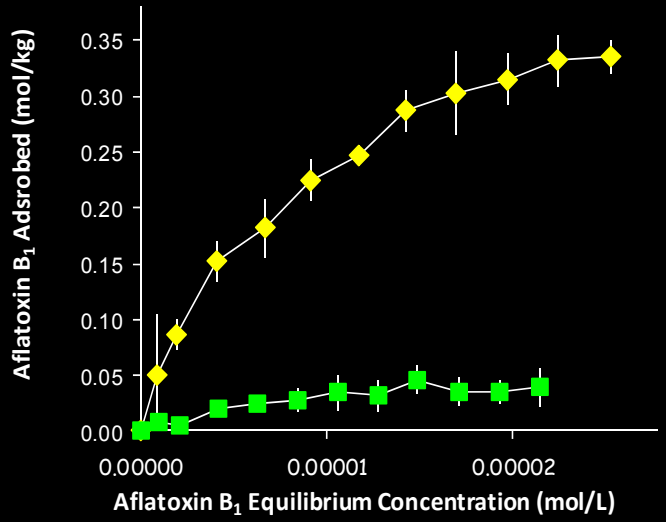
- Grind and sieve (-325 mesh)

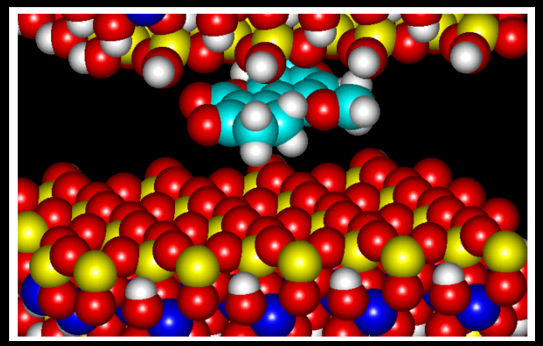


- React sorbent and toxin; incubate 24 h

- UV detection of AfB_1; Data processing

- Curve fitting and derivation of Qmax, Kd and enthalpy





Molecular modeling

AFLATOXIN ADSORPTION ISOTHERMS

Langmuir model:

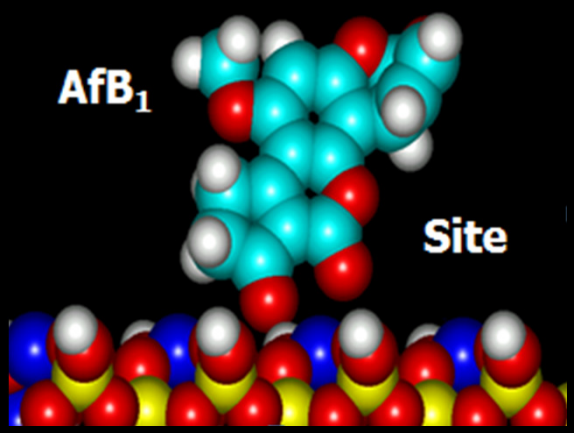
$$q = Q_{max}(K_d.C_w/1 + K_d.C_w)$$

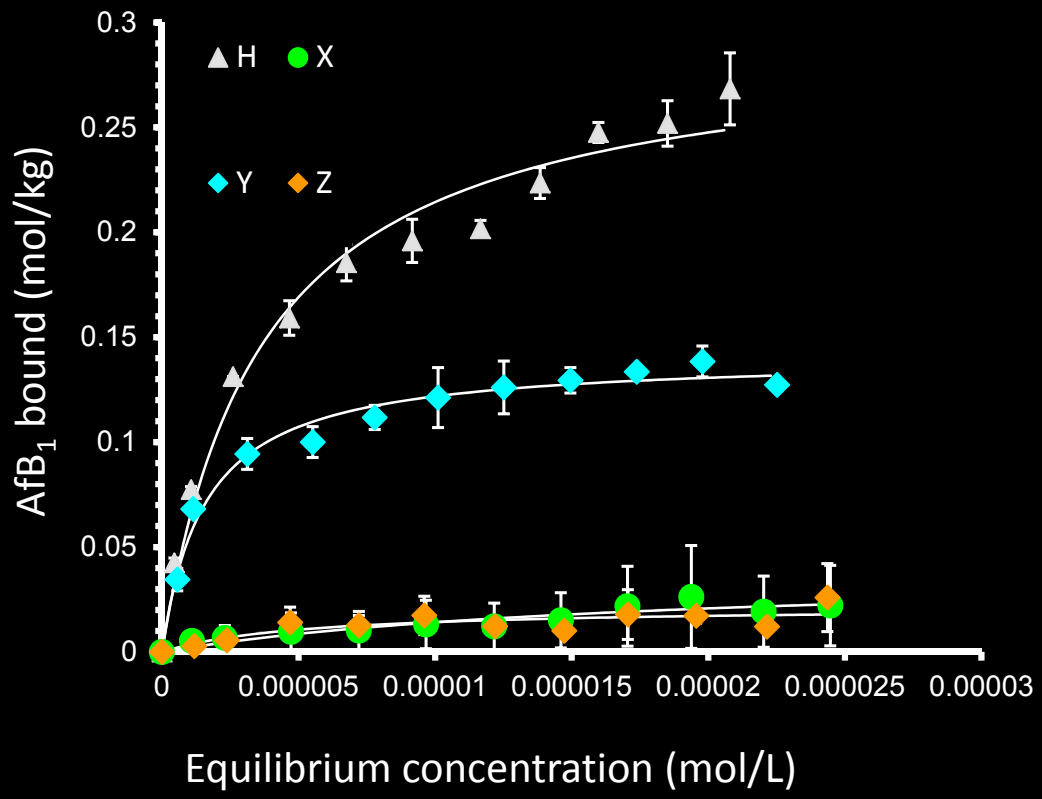
can be rearranged as:

$$K_d = q/(Q_{max} - q).C_w$$

where:

$$\underset{C_w}{\text{ligand}} + \underset{Q_{max}-q}{\text{site}} \overset{K_d}{<->} \underset{q}{\text{ligand-site}}$$

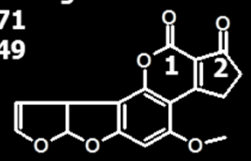




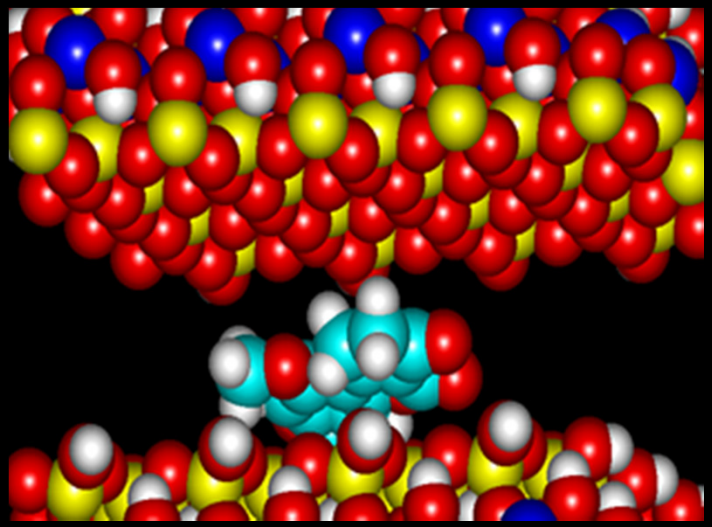
Meaningful derivations: Q_{max}; k_d, enthalpy (kJ/mol); plateau surface density; number of sites available; type of binding (monolayer coverage, etc.)

SUMMARY OF OUR *IN VITRO* RESULTS

	Partial Charge
1	0.271
2	0.149



Aflatoxin B_1 MW = 312.3



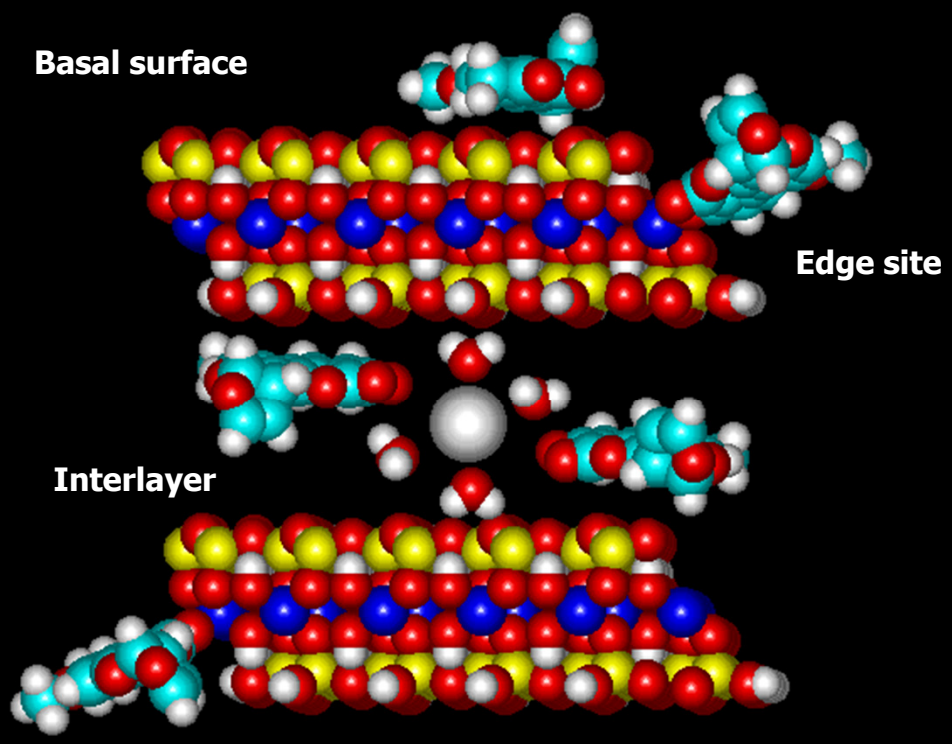
Criteria for sorption and efficacy

1. Planar dicarbonyl group with a bridgehead double bond and partially positive carbons that are available for electron donor-acceptor reactions (DRIFTS-IR)
2. Favorable thermodynamics, heat of sorption (ΔH) $\geq$ -20 kJ/mol.
3. Saturable, high affinity sorption: Qmax > 0.25 mol toxin/kg sorbent; Kd $\geq 10^5$
4. Plateau surface density $\geq$ 305 A^2/molecule
5. Groups that become cationic at acid pH

Critical Characteristics of Sorbent

1. Dioctahedral Smectite (Intact gallery)
2. Lack of interaction with nutrients
3. Tested for safety in multiple animal species

MODEL FOR SORPTION ONTO THE SURFACE OF CLAY



SUMMARY:

- Greater than 90% of aflatoxins are bound in the interlayer of NS, with the remainder at basal and edge sites
- Sorption of aflatoxins exhibits both stereo- and regio-specificity
- Thermodynamics of the surface interaction between aflatoxins and NS are favored, with an enthalpy of sorption between -40 to -50 kJ/mol
- NS has a preference for aflatoxins with high capacity and high affinity for sorption to interlayer surfaces

ANIMAL STUDIES WITH NS CLAY

From 1988 through 2016, forty nine (49) studies have reported the safety and efficacy of NS clay in chickens, turkeys, pigs, dogs, lambs, cows, goats, fish, mice and rats. These studies support the *in vitro* work with NS clay.







SUMMARY OF ANIMAL STUDIES WITH NS

- **No adverse health effects from consumption of NS and strong evidence for aflatoxin preference in animals. NS did not interfere with the utilization of vitamins and minerals in long-term studies in rats. Importantly, NS inclusion in the diet protected against levels of aflatoxins in young animals as high as *7,500 ppb. This level is comparable to "lethal" levels of aflatoxins from contaminated maize in outbreaks in Kenya (2004).**






CURRENT USE OF NS: MITIGATE AFLATOXICOSIS IN ANIMALS DURING OUTBREAKS

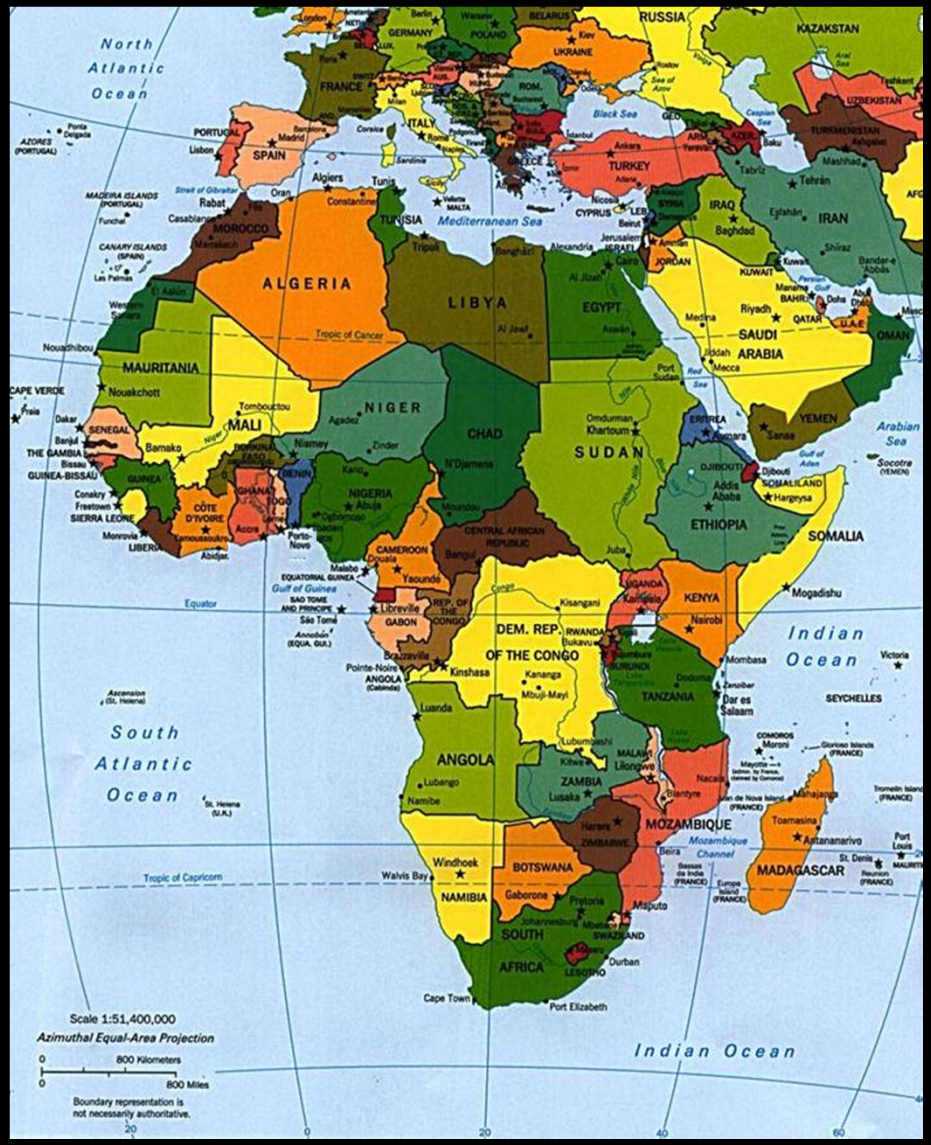
PARTICIPANTS: Feed Producers and Processors, Poultry and Livestock Industries, Dairy and Equine Industry, Aquaculture Industry, Farms and Ranches

MY MAIN OBJECTIVE: To translate the NS strategy used for animals to people and communities, especially infants and children who are the most vulnerable during aflatoxin outbreaks.

HUMAN STUDIES WITH NS CLAY

From 2005 through 2016, a total of six clinical intervention trials in Texas, Ghana, and Kenya have confirmed the safety and ability of NS clay to reduce biomarkers of aflatoxin exposure, supporting our *in vitro* and animal work.

Phase IIa Clinical Trial with NS in Ghana, West Africa



Study Site: Ejura-Sekyedumase district, one of the 21 districts in the Ashanti Region

Preliminary data showed 4 communities had high aflatoxin exposure: 100% of participants were positive for AfB1-albumin adducts; >90% were positive for AFM1 metabolites

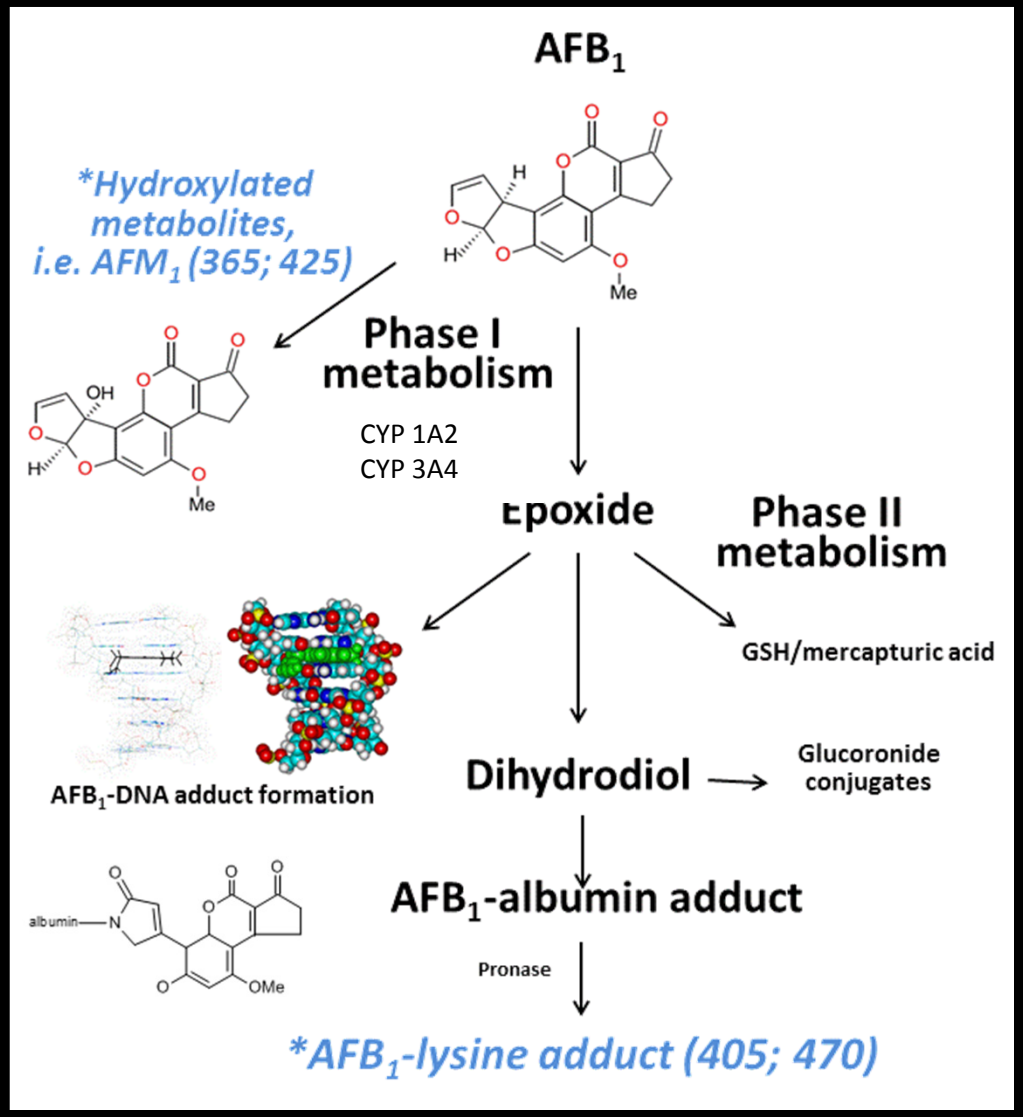
AFLATOXIN EXPOSURE BIOMARKERS

AFB_1 metabolism produces a series of potential biomarkers of exposure in blood and urine. We have used 2 of these to evaluate exposures and the efficacy of clay interventions.

Urinary Biomarker: Aflatoxin M_1 in urine is measured using immunoaffinity cleanup and HPLC with fluorescence detection and LC-MS/MS

Serum Biomarker: Aflatoxin B_1-lysine (derived from the albumin adduct) in blood serum can be measured by HPLC and LC/MS-MS.

*The lysine adduct is stable but requires > 2 wks intervention to detect differences; the urine metabolite represents exposure the day before.

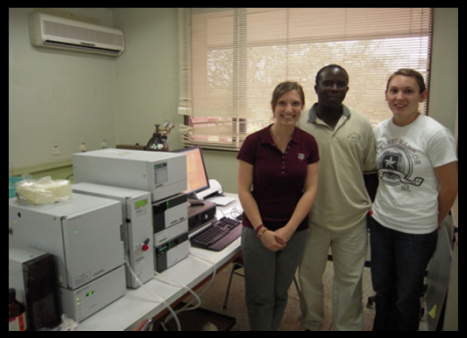


Dietary exposure to aflatoxins from maize in the Northern Ashanti region of Ghana

COMMUNITY ENTRY AND CAPACITY BUILDING

- Technology transfer to our laboratory at the Noguchi Medical Research Institute in Accra and at KNUST University in Kumasi; community entry at Nkwanta

Induction ceremony



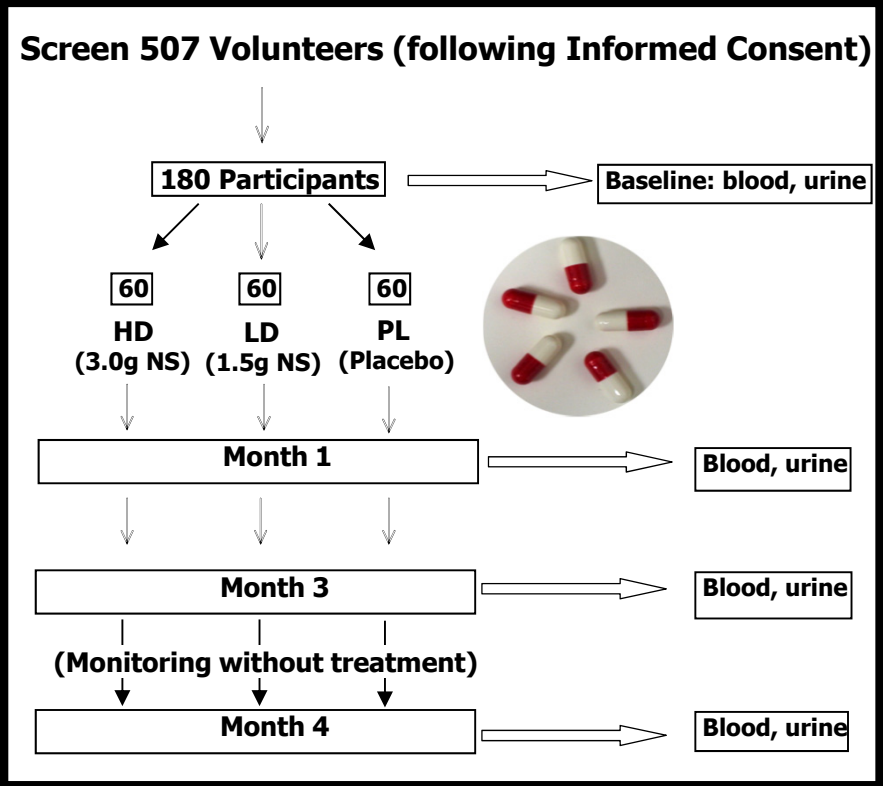
Phase IIa Clinical Trial with NS in Ghana

Following an initial Phase I dosimetry study in Texas

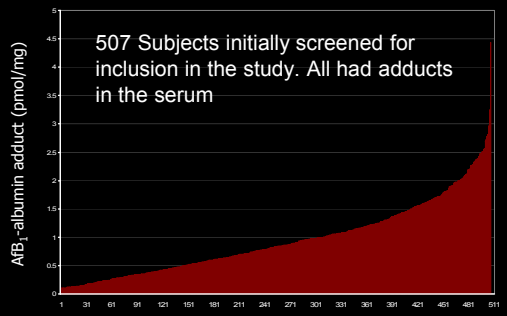
Hypothesis: NS clay will act as an enterosorbent for aflatoxins resulting in reduced bioavailability from the diet.

Study Design: Randomized, double blind, placebo-controlled Phase IIa clinical intervention trial

Study monitors and colleagues in Ejura



Distribution of Aflatoxin Adducts in the Ashanti Region of Ghana



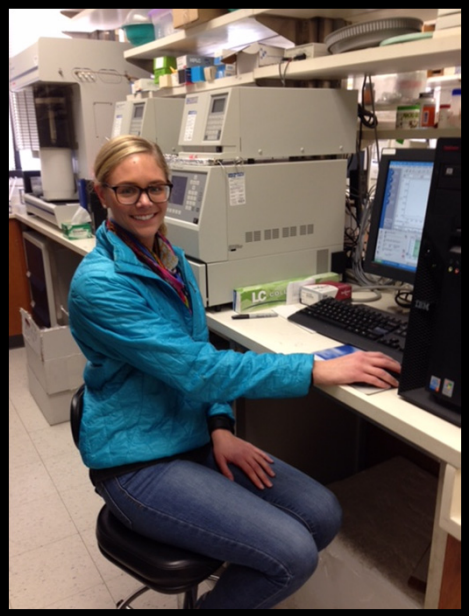
180 participants: Healthy subjects age 18-59; no history of chronic disease; intake of corn and/or peanuts at least 4 times/week; positive for serum biomarkers.

FURTHER STUDIES

AT SITES IN GHANA, KENYA AND SAN ANTONIO (A total of 6 clinical trials)

AIM: Confirm the safety and potential efficacy (based on exposure biomarkers) of NS clay in adults and children

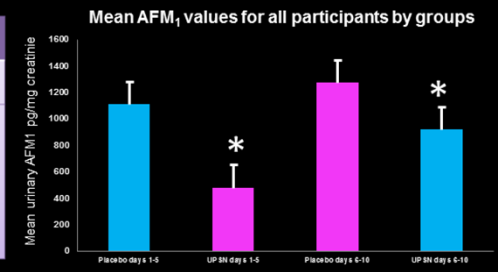
Approach: Delivery of a refined NS in food in Ghana. Hypothesis: Clay can be included in common foods/snacks or liquids during outbreak periods (e.g., added into corn flour at the community mill when needed).

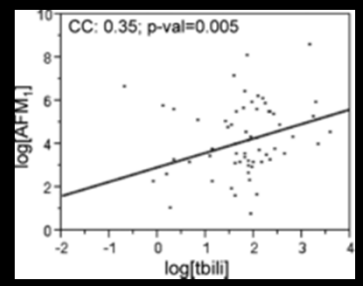


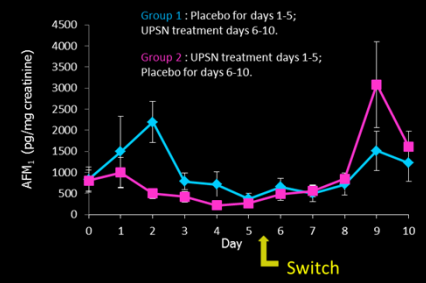
SUMMARY OF FINDINGS

- **CAPSULES:** A 3-month study in Ghana indicated that NS and refined NS were safe for consumption, with minimal adverse effects and good compliance. The clay significantly reduced blood and urine biomarkers. **FOOD:** In a 2-wk cross-over study (food), clay did not alter the taste, aroma, or texture of food, and significantly reduced aflatoxin M_1 in urine samples by 55% compared to the placebo.

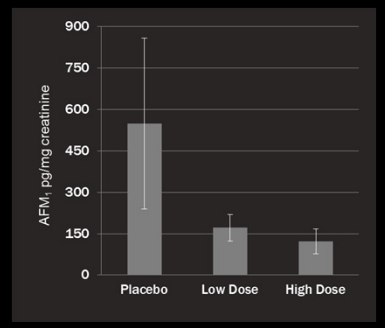
Palatability of foods containing 1.5 g of calcium carbonate or UPSN clay twice/day				
	Taste	Aroma	Texture	Eat Again?
Good	84.8% 78.3%	78.3% 65.2%	80.4% 69.6%	YES
Acceptable	15.2% 21.7%	21.7% 34.8%	19.6% 30.4%	
Unacceptable	No food samples were deemed to be unacceptable or poor			
Poor				



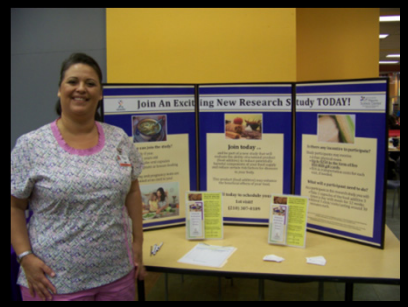




- **FOOD:** A 2-week study with ¼ and ½ dose of NS in 63 children (ages 3-9) at our site in Ghana did not result in treatment-related adverse events; the dose group showed a 52% reduction in median AFM_1 levels compared with control.



- **CAPSULES:** Recent study in TX; same protocol as the study in Ghana (using capsules) confirmed the safety of clay. Although, a dose-dependent reduction of adduct was not observed, the low dose of clay significantly reduced adduct levels by 36%. Completion: 63%. Aherence: 85%

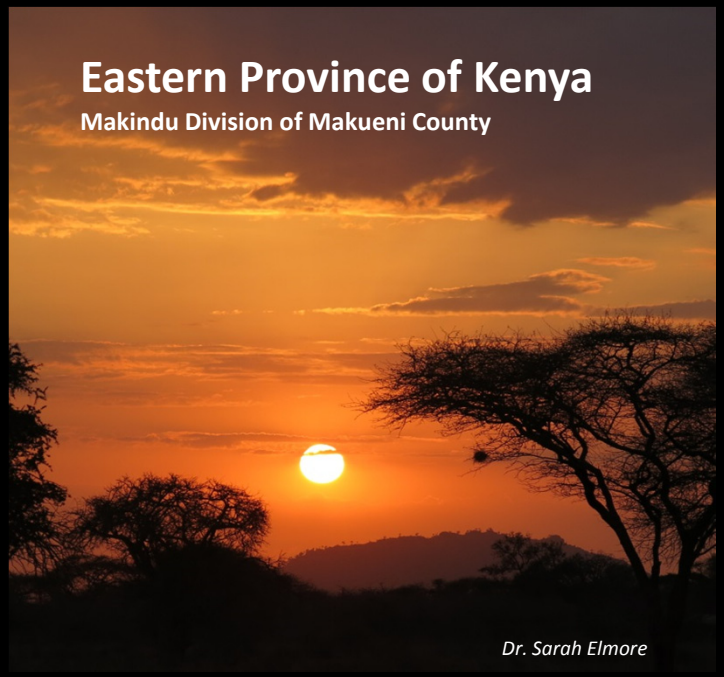


PARTICIPANT COMPLIANCE & COMPLETION OF TREATMENT REGIMEN

	Treatment group			
	High dose	Low dose	Placebo	Overall
Participants				
Randomized	71	83	80	254
Completed (3 mo. treatment)	44	51	52	147
Completion (%)	62.0	61.4	65.0	62.8
Treatment Regimen				
Capsules to be ingested	23688	27888	27216	78792
Capsules missed (reported at V2 and V4)	3236	3967	4504	11707
Capsules taken (reported at V3 and V4)	20452	23921	22712	67085
Total reported adherence (%)	86.3	85.8	83.5	85.1

RECENT STUDY

In collaboration with the CDC: 2-Week Cross-Over with delivery of NS from sachets in flavored water



50 participants (25/group: placebo or treatment). 7 days treatment-switch and 5 days washout-7 days treatment. Baseline urines and blood, daily urines; final urines and blood at termination of the study.

FINDINGS: Clay reduced levels of AFM_1 in urine and serum adduct up to 46%. Palatability and acceptability were excellent. They would be willing to take the clay during an outbreak and give it to their children.

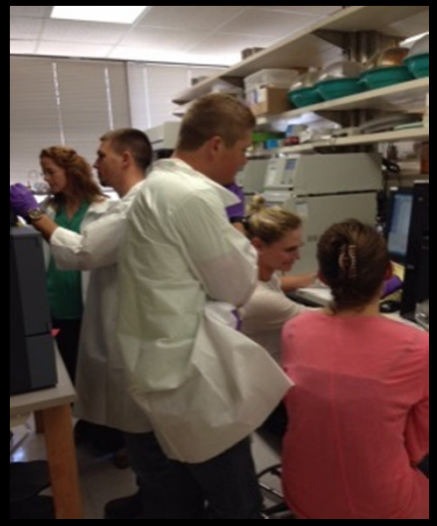
Potential Benefits of NS Clay

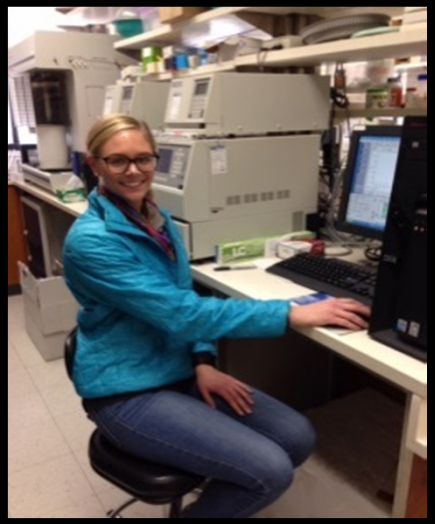
1) Short-term inclusion of clay in the diet to reduce the effects of aflatoxins and to prevent mortality and morbidity in infants, children and young animals during outbreaks and emergencies.

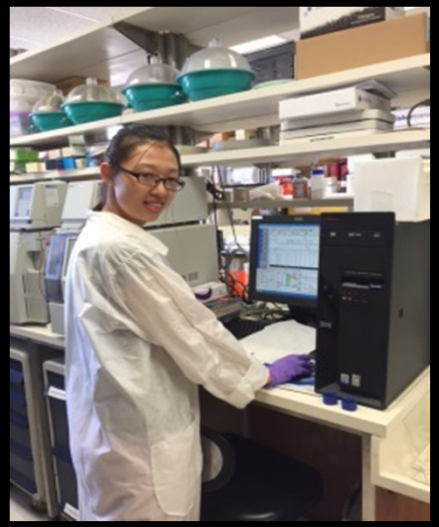
2) Possible spin-off applications for the treatment of diarrhea in infants and from cancer chemotherapy and enteric complications for individuals living with chronic illnesses such as inflammatory bowel disease and other gastrointestinal conditions

3) New paradigms for the enhancement of food quality, food safety and food security in developing countries, e.g. utilize community millers to incorporate NS into maize flour and snacks during aflatoxin outbreaks; Include NS in nutritional supplements (containing contaminated corn and peanuts) to neutralize aflatoxins during outbreaks; Include NS in liquids, e.g. flavored water, tea, juice, etc.

Phillips Laboratory







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