Managing Risks Associated With Feeding Aflatoxin Contaminated Feed

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Summary

Aflatoxin contamination of foods and livestock feeds is an ongoing problem that is magnified with the occurrence of drought. The toxic effects (and risks) associated with aflatoxin exposure can be mitigated with dietary inclusion of a clay-based toxin enterosorbent. A calcium montmorillonite clay that is commonly used as an anticaking agent in feeds (i.e., NovaSil^{TM} **[NS]**) has been evaluated for its ability to bind aflatoxin B_1 (AFB_1) *in vitro* and *in vivo* to prevent aflatoxicosis in animals and humans. In addition, decreasing the bioavailability of AFB_1 results in significantly less AFM_1 transfer to milk. Isothermal analyses conducted with NS and AFB_1 to quantitate and characterize critical sorption parameters at equilibrium, (i.e. ligand saturation capacities, affinity constants, and thermodynamics of the sorption process) indicate that AFB_1 is tightly sorbed onto the surface of NS, which exhibits high capacity and high affinity for the ligand. Thermodynamics favor sorption of AFB_1 to NS. The process is exothermic and spontaneous with a mean heat of sorption equal to approximately -50 kJ/mol, suggesting chemisorption (or tight binding). Dietary NS inclusion at levels as low as 0.5% significantly protects animals and (potentially) humans from the effects of high level exposure to aflatoxins and does not significantly interfere with vitamin or mineral uptake.

Introduction

Aflatoxin origin and congeners

Aflatoxins **(AF)** are harmful secondary metabolites of mold growth that can cause disease and death in both animals and humans (CAST, 2003). Aflatoxins, produced by the common fungi *Aspergillus flavus* and *A. parasiticus*, are heat stable and resistant to a wide variety of food processing methods. Aflatoxins were first identified in the United Kingdom in 1961, where highly contaminated animal feed was responsible for the death of 100,000 turkeys (Sargeant et al., 1961). Staple foods that are highly susceptible to AF contamination include maize, peanuts (Phillips et al., 2006), cottonseed, and tree nuts (International Agency for Research on Cancer **(IARC)**, 2002). There are 4 major AF congeners that occur as direct contaminants of foods and feeds: aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1) and aflatoxin G_2 (AFG_2) (Figure 1). Additionally, the aflatoxin M_1 (AFM_1) metabolite, which is hydroxylated at the 9a position, is commonly found in the milk of lactating animals consuming AFB_1 contaminated diets.

Occurrence

Aflatoxins contaminate a variety of agricultural commodities, but they are most frequently found in maize, peanuts, cottonseed, and tree nuts. Their occurrence is worldwide, but a

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higher prevalence of AF is typically found in tropical/semitropical regions. Geographical distribution of AF correlates well with climate conditions that promote *Aspergillus* growth, which include drought and humidity. Global climate change may be responsible for the recent emergence of AF problems in crops grown in regions that previously had a low incidence of AF outbreaks. Under favorable pre-harvest conditions, *A. flavus* and *A. parasiticus* can produce AF in developing seeds of corn, peanuts, cotton, almond, pistachio, and other tree nuts (Bhatnagar et al., 2002; CAST, 2003). However, the production of AF depends on many environmental and nutritional factors, including moisture, temperature, substrate, aeration, pH levels, quantity of carbon and nitrogen sources, lipid content, concentration of metal salts, and specific nutrient requirements (Ominski et al., 1994; Cary et al., 2000; CAST, 2003). Water activity **(a_w)** is an important factor for pre-harvest contamination, where values of $a_w \geq 0.85$ have been shown to result in increased contamination of susceptible crops (Wilson and Payne, 1994). Contamination with AF can also occur during storage after harvest and is dependent upon certain environmental conditions, such as temperature and moisture. Specifically, temperatures ranging from 77 to 93°F (Wilson and Payne, 1994) and moisture levels exceeding 7% (Williams et al., 2004) are the main factors that influence postharvest storage contamination.

Metabolism

Aflatoxins must be bioactivated in order to exert their toxic and carcinogenic effects. Extensive research has focused on biotransformation pathways for AFB_1, specifically, since it is the most prevalent and carcinogenic of the AF. Aflatoxin B_1 metabolism includes major biochemical processes, including oxidation (Essigmann et al., 1977, Groopman, 1994), reduction (Wong and Hsieh, 1978), hydroxylation (Moss and Neal, 1985), and conjugation (Holeski et al., 1987). Following absorption through the gastrointestinal wall, AFB_1 enters the liver through the portal vein and a portion is then distributed to soft tissues. Nevertheless, the majority of AF accumulate in the liver and kidneys, where most biotransformation occurs (Leeson et al., 1995). In the human liver, AFB_1 is primarily metabolized by CYP3A4 and CYP1A2 enzymes, leading to the formation of AFB_1 *exo*-8,9-epoxide and *endo*-8,9-epoxide (Gallagher et al., 1994; Guengerich et al., 1998). Due to its instability, the more abundant *exo*-epoxide covalently binds to DNA, commonly at the N-7 position of guanine (Essigmann et al., 1983; Gopalakrishnan et al., 1990). Aflatoxin B_1 can also undergo hydroxylation, resulting in the formation of AFM_1 and AFQ_1 (Ramsdell and Eaton, 1990), or demethylation, which results in the production of AFP_1 (Wong and Hsieh, 1980; Eaton et al., 1994). Additionally, lipoxygenases and prostaglandin H synthase may also play a significant role in extra-hepatic organ AF metabolism (Battista and Marnnet, 1985; Liu and Massey, 1992). Metabolic phase I enzymes, such as epoxide hydrolases **(EH)**, are responsible for the conversion of the epoxide to dihydrodiols, while phase II enzymes, such as glutathione S-transferase **(GST)**, increase the solubility of the epoxide by forming GSH conjugates. These products are eventually excreted in urine (Hayes et al., 1993; Wild and Turner, 2002), although a small portion may remain in tissues and body fluids, such as blood (Leeson et al., 1995). Species-specific differences in AF metabolism account for the variations in toxicity and carcinogenic effects observed in animals and humans.

Aflatoxin M_1

The AFM_1 mainly occurs in milk and milk derived products; however, the quantification of this metabolite in urine is well documented in humans and animals as a linear biomarker of exposure to AFB_1, (Jolly et al., 2006; Phillips et al., 2008). Of the cytochrome P450 enzymes, CYP1A2 is mainly responsible for oxidizing AFB_1 into the AFM_1

metabolite (Eaton et al., 1994; Wang et al., 1999). Even though AFM_1 is less toxic than the parent compound, it is still considered a public health concern due to the high consumption of milk and milk-based products by humans, especially children. Regulatory agencies, such as the Food and Drug Administration **(FDA)** and the European Food Safety Authority **(EFSA)** have established maximum permissible levels of 0.5 μg kg^{-1} (ppb) and 0.05 μg kg^{-1} (ppb) AFM_1 in milk, respectively. However, the EFSA regulates this metabolite more strictly (0.025 μg kg^{1} ppb) for infant formulas. To comply with these requirements, strict concentration limits have been established for AF in feed ingredients used for dairy cows. For example, the AF concentration in feed has been restricted to 20 μg kg^{-1} (ppb) in the U.S.

AF Toxic Effects

Children and the young of all animal species are the most susceptible to the toxic effects of AF. The International Agency for Research in Cancer **(IARC)** considers AFB_1 and mixtures of AF as group 1 carcinogens, while AFM_1 is listed as a group 2B carcinogen. Jaundice, fever, ascites, vomiting, and edema of the feet have been observed in documented cases of aflatoxicosis in India, in addition to fatality, which occurred in people who consumed 2 to 6 mg of aflatoxin daily for one month (Krishnamachari et al., 1975). In Kenya, centrolobular necrosis of the liver was associated with consumption of AFB_1-contaminated maize at levels up to 12 mg kg^{-1} (ppm) (Ngindu et al., 1982). Aflatoxins have also been associated with the development of cirrhosis (Amla et al., 1971) and kwashiorkor (Hendrickse and Maxwell, 1989), as well as encephalitis and fatty degeneration of the viscera (Shank et al., 1971).

Cases of aflatoxicosis have been reported in a variety of agriculturally important animals. Some important outbreaks in poultry have occurred in India (Choudary and Rao, 1982; ICRISAT, 2002), where high mortality was observed. The swine industry has also been affected; an outbreak was reported in Sentinela do Sul, RS, Brazil in 2004, in which apathy, anorexia, jaundice, dark urine, hematuria, and photosensitization were among clinical signs observed (Zlotowski et al., 2004). Necropsy revealed generalized jaundice, discolored liver, edema of the gall bladder wall and yellowish effusion into the abdominal and pericardial cavities. Although bovine are more resistant to AF effects than some species, they can also be affected. In 1985, more than 200 head of young cattle died from aflatoxicosis following ingestion of feed contaminated with concentrations of AF ranging from 96 to 1700 μg kg^{-1} (ppb) (Osweiler and Trampel, 1985). In that study, diagnosis was based on AF concentrations in cottonseed feed products, as well as macroscopic findings in the liver and detection of AFB_1 and AFM_1 in urine and liver from affected calves.

Although the liver is the predominant target organ susceptible to AF toxicity and carcinogenicity (Miller and Wilson, 1994), other organs and biological systems can be affected (Coulombe, 1994). This appears to be the case for the immune system, where AF have been shown to exert suppressive effects (Bondy and Pestka, 2000). Induction of thymic aplasia (Pier, 1986), reduction of T-lymphocyte number and function, suppression of phagocytic activity, decreased complement activity (Richard et al., 1978; Pier, 1986; Reddy et al., 1987) and modifications in cytokine secretion and interleukin gene expression (Han et al., 1999; Moon et al., 1999) are among the immuno-suppressive effects exerted by AF in animals. Data supporting the occurrence of immunomodulation after AF exposure in humans is less abundant; however, it is possible that the progression of immune system related diseases could be hastened by frequent AF ingestion, due to the fact that high levels of AF-albumin adduct in human immunodeficiency virus **(HIV)**-infected individuals result in significantly lower levels of T-cells and □-cells compared to those with low adduct levels (Jiang et al., 2008).

Among various strategies that have been successfully utilized to reduce animal and human exposures to mycotoxins, the binding of aflatoxins in the gastrointestinal tract using refined montmorillonite clays appears to be a safe and viable approach (Phillips, 1999; Phillips et al., 2008) and has the potential to benefit more than 4.5 billion people and their animals residing in regions of the globe susceptible to AF exposure (Williams et al., 2004).

Reducing Risks of Aflatoxin Exposure

Mitigation with clays

Strategies for the reduction of AF include thermal inactivation, irradiation, solvent extraction, mechanical separation, density segregation, adsorption from solution, microbial inactivation, chemical inactivation, chemoprevention, and clay therapy (Kensler et al., 1994; Phillips et al., 1994). Of these methods, the latter 3 are the most practical for feeds and foods. The ingestion of clay-based materials by animals and humans (i.e., geophagy) has been practiced throughout history. The use of clays has been reported to occur as early as 2,000 years ago for alleviation of diarrhea and skin infections, and to bind toxic substances. Clays are used by man and animals on many continents and are widely used as traditional medicine in many African countries and in China (Johns and Duquette, 1991; Diamond, 1999). Today, the inclusion of clay-based products in feed is a strategy frequently utilized to reduce AF exposure in animals. Natural bentonites and montmorillonites are common sorbents used for this purpose. Pioneering studies in the Phillips laboratory at Texas A&M University indicated that low levels of calcium montmorillonite clay strongly and preferentially sorb AF. In further work with scientists at the USDA/ARS, low levels of NS clay, when included in the diet, significantly reduced the bioavailability and adverse effects of AF exposure in a variety of animals (Phillips et al., 1988; Phillips, 1999). A refined version of NovaSil clay **(NS)** has been shown to decrease the level of AFM_1 in milk from lactating dairy cows and goats (Harvey et al., 1991b; Smith et al., 1994).

NS physical characteristics and activity

Bentonite is defined as a relatively impure clay consisting primarily of montmorillonite, the most abundant mineral found among members of the smectite group, which also includes beidellites, nontronites, hectorites, saponites, and sauconites. Smectites are unique, 2:1 layered minerals that exhibit expansive properties and a net negative interlayer charge (Figure 2). The principal source of charge in smectites arises as a result of isomorphic substitution. In montmorillonites, the substitution of Mg^{2+} for Al^{3+} in the octahedral layer produces this characteristic negative charge. These minerals are well known for their small particle size of $< 2\mu m$, and their high internal surface area of 600 to 800 $m^2 g^{-1}$, which allows for high capacity sorption.

The NS consists of a modified calcium bentonite clay, which is naturally rich with montmorillonite minerals. Montmorillonites crystallize to form dioctahedral smectites, which comprise the active component of NS. Compositional analysis indicates that NS consists of 0.2% free iron oxides, 1% sand-sized particles ($> 50\ \mu m$), 22.7% silt-sized particles (2 to 50 μm), >74.0% clay-sized particles ($\leq 2\ \mu m$), and 2.1% mica. Montmorillonite also exists in the sand and silt fractions of NS, which is likely a result of incomplete disaggregation of individual clay particles. Scanning electron microscopy **(SEM)** confirms that the silt fraction of refined NS contains smectite, in addition to feldspars and mica (Figure 3a). The most noteworthy feature observed by transmission electron microscopy **(TEM)** is the folding tendency of montmorillonite particles (Figure 3b). The particles contain thin films of considerable length (3 to 5 μm) and resemble veil fabric. The x-ray defraction **(XRD)** patterns of the clay fraction

indicate that montmorillonite is the only mineral present in this component. The pH of NS was calculated to be between 7 to 10, which is well within normal range for montmorillonites. The cation exchange capacity **(CEC)** of the clay fraction (82.9 cmol/kg) further confirms the montmorillonite identity. Comparison of extractable bases using ammonium acetate (NH_4OAc) revealed that the Ca^{2+} cation was present in the highest concentration in NS (85.8, 9.1, 0.5, and 1.5 cmol kg-1 for Ca, Mg, Na, and K, respectively). Fourier-Transform Infrared Spectroscopy **(FTIR)** analysis further verified the montmorillonite nature of NS, and more importantly, it showed evidence of Fe and Mg isomorphic substitution in the octahedral layer which is characteristic of effective AF binders.

Characterization of NS binding interactions

To help characterize the mechanisms of aflatoxin sorption onto the surfaces of NS, isotherms have been performed as previously described by Grant and Phillips (1998). In this assay, a stock solution of AFB_1 is prepared by dissolving pure crystals in acetonitrile and then injecting a volume from the dissolved AFB_1 into purified water to yield a solution concentration of 8 mg ml^{-1}. The concentration is further verified by measuring the absorbance at 362 nm on a ultraviolet **(UV)**-visible spectrophotometer. Isothermal analyses for each additive are conducted at pH values of 2 and 6.5 to simulate stomach and small intestine pH conditions, respectively. The pH of the solutions used is adjusted with concentrated hydrochloric acid and sodium hydroxide. An isotherm consists of 11 concentrations of AFB_1 (0.4, 0.8, 1.2, 2.4, 3.6, 4.0, 4.8, 5.6, 6.4, 7.2 and 8.0 mg ml^{-1}) mixed with 0.1 mg of NS in a total volume of 5 ml in borosilicate glass tubes. Three replicates are used for each solute concentration. The suspension is mixed to ensure slurry homogeneity. From this suspension, 50 ml (0.1 mg of NS) is added to each of the dilution tubes and the suspensions are vigorously stirred during the addition of the NS. Controls consist of 5 ml of water, 5 ml of AFB_1 stock solution, and 5 ml of water with 0.1 mg of NS. The samples and controls are shaken at 1000 rpm for 2 h in an incubator at 25°C, followed by centrifugation at 2000 rpm for 20 min. The supernatant is then measured for absorbance at 362 nm. The UV adsorption data are used to calculate the amount of AFB_1 left in solution at equilibrium (C_w) and the amount adsorbed to the clay (q) using Table Curve 2D v.2 software and an Excel program developed in the Phillips laboratory to fit the data to a standard Langmuir-derived isotherm equation:

$$q = Q_{max}\left(K_d C_w / 1 + K_d C_w\right)$$

The binding capacity (Q_{max}) and the distribution constant (K_d) can be determined for NS using the Grant and Phillips (1998) method. The adsorption of AFB_1 onto dioctahedral smectite clays can be classified as Langmuir 1 or 2 curves. In this pattern, the sorbent is reaching saturation or saturation has been reached as the concentration of the solute is increased. It has been reported that L type curves are commonly depicted by chemisorption mechanisms between adsorbates and adsorbents (Adamson, 1982). Initial strong adsorption followed by a plateau in an isothermal plot suggests a specific type of binding with subsequent saturation of that type of binding site (Grant and Phillips, 1998).

Different sites of AFB_1 adsorption onto smectite surfaces have been proposed and include external surfaces, edge surfaces (Desheng et al., 2005), and interlayer spaces (Phillips et al., 2002; Kannewischer et al., 2006). Data supporting interlayer adsorption were first presented by Grant and Phillips (1998). In that study, a hydrated sodium calcium aluminosilicate (HSCAS or NS) was heated at 800°C for 1 hour to collapse the interlayer area, resulting in significant reduction of binding capacity and demonstrating the importance of the interlayer as an adsorption site (Figure 4). There are also numerous proposed sorption mechanisms that could

be occurring at the active binding sites, including selective chemisorption, an electron donor acceptor **(EDA)** mechanism, hydrogen bonding, furan ring bonding, ion dipole interactions, and coordination between the exchange cations and carbonyl groups.

Using simulated molecular models, Phillips et al. (1995) proposed that AFB_1 can be sorbed at the edges, the interlayer surfaces, and the basal surfaces of smectite by preferential chemisorption. Evidence for chemisorption was collected through a series of experiments, which concluded that the carbonyls from the lactone and cyclopentenone rings of AF formed chelates with transition metals in the clay surfaces and suggested the formation of a mononuclear (bidentate) chelate with the transition metals contained in the clay (Phillips et al., 1995).

The electron donor mechanism was proposed in later studies, based on experiments that calculated the enthalpy (ΔH) of adsorption. Phillips (1999) documented an enthalpy value of -40 kJ mol^{-1} for AFB_1 bound to the surfaces of montmorillonite clay. In this study, it was proposed that the partially positive carbon atoms of the 2 carbonyl groups on the AF molecule share electrons from the negative siloxane surfaces of the clay. Additionally, a positive correlation between the magnitude of partial positive charges on the carbons C11 and C1 of the β-dicarbonyl system of planar analogs and derivatives of AFB_1 versus the strength of adsorption was observed. The hydrogen bonding mechanism for AF binding to clay has been reported by (Desheng et al., 2005), who proposed that formation of double hydrogen bonds between AF, and the edge sites of montmorillonite is the main reaction mechanism of sorption. Additionally, Tenorio-Arvide et al. (2008) proposed that epoxidation of the dihydrofuran ring may occur due to smectite-AFB_1 interactions and further suggested that this epoxidation may contribute to the bonding of the toxin to smectites. The chelation of AFB_1 with Ca^{2+} and other cations or edge-site metals was also proposed as a potential mechanism for binding (Phillips, 1999). Additionally, infrared analyses revealed that exchangeable cations in the interlayer may coordinate with the 2 carbons of the coumarin moiety (Tenorio-Arvide et al., 2008). Recently, more specific characterization of AFB_1-smectite binding suggests that the mechanisms involved can vary based on hydration conditions (Deng et al., 2010), with ion-dipole interactions and coordination between exchangeable cations and carbonyl groups being of major importance under dry conditions and H-bonding between carbonyl groups and exchangeable-cation hydration-shell water being the predominant forces involved under high moisture conditions. Importantly, it is possible that these mechanisms may be occurring simultaneously, which may account for the high capacity of smectite clays to bind AF.

NS efficacy in vivo

The utilization of calcium montmorillonite clays (e.g., NS) to tightly sorb and inactivate AFB1 in the gastrointestinal tract of various animal species has shown significant promise in mitigating the effects of AF. Several studies have demonstrated that dietary inclusion of NS prevented aflatoxicosis in multiple animal species, including rodents (Mayura et al., 1998), chickens (Phillips et al., 1988; Kubena et al., 1990b), turkeys (Kubena et al., 1991), dogs (Bingham et al., 2004), swine (Lindemann et al., 1993), lambs (Harvey et al., 1991a), goats (Smith et al., 1994), and dairy cattle (Harvey et al., 1991b). In addition, the clay (at 1.0% level in feed) significantly reduced AFM1 concentrations in milk without altering the nutritional quality or causing overt toxicity itself (Harvey et al., 1991b).

The AFB_1 metabolism also produces a series of potential exposure biomarkers in the blood and urine, 2 of which have been used in previous and ongoing animal and human studies in our laboratory (Figure 5). Since determination of AF concentrations in food and feeds can be confounded due to a lack of uniform distribution of the toxin, the

analysis of biomarkers is critical in order to gain an accurate understanding of the extent of dietary exposure in humans and animals. Urinary AFM_1 is an acute exposure biomarker and is indicative of exposures within the previous 48 hr, whereas the measurement of serum aflatoxin B_1-albumin adduct (AF-alb), with a half-life of ~21 days, is typically utilized to obtain chronic exposure data. Currently, the AF-alb assay is heavily relied upon for human exposure and mitigation studies, but it has yet to be developed for AF exposures in livestock; however, this capability would likely be of great value for increasing herd health and for litigation purposes. Furthermore, the ability to detect both AFM_1 and AF-alb are valuable in the determination of sorbent efficacy.

NovaSil at an inclusion level as low as 0.5% in the AF-contaminated diet of sensitive animal species effectively reduced the health effects of aflatoxicosis (Phillips et al., 1995). In previous research, NS diminished the acute effects of AFB_1, as evidenced by the reduction of AFM_1 concentrations in the urine of AF-exposed rats (Sarr et al., 1995). Preference of NS for AF has been well-characterized in several animal studies. For example, NS was shown to be ineffective for sorption of diacetoxyscirpenol **(DAS)** (Kubena et al., 1993), ochratoxin A **(OA)** (Huff et al., 1992), or T-2 toxin (Kubena et al., 1990a) in chicks, and for deoxynivalenol **(DON)** in pigs (Patterson and Young, 1993). However, when NS was combined with all of these mycotoxins, NS effectively protected against aflatoxicosis, confirming the preference of this clay for AF. NovaSil Plus **(NSP)**, which is structurally similar to NS, has also been shown to protect animals from the effects of AF. In a previous study, male Fisher-344 rats fed diets containing AFB_1 (dissolved in corn oil) and 0.5% NSP, as previously described (Mayura et al., 1998), exhibited a 95% reduction of urinary AFM_1 compared to rats dosed without NSP (Bingham et al., 2004). In a separate study in dogs, it was determined that dietary inclusion of 0.5% NSP also reduced AFM_1 concentrations by an average of 48.4% compared to dogs fed a diet without NSP (Bingham et al., 2004). Furthermore, concentrations as high as 2% NSP in the diet throughout pregnancy produced neither maternal nor fetal toxicity in Sprague-Dawley (S-D) rats and did not increase metal bioavailability in a variety of tissues (Wiles et al., 2004).

The NSP clay also shows promise for AF mitigation in humans, with significant reduction of blood and urine biomarkers (Afriyie-Gyawu et al., 2008a). Importantly, carefully designed Phase I and II clinical trials in Texas and Ghana have confirmed that it is safe for human consumption and does not elicit significant adverse effects (Wang et al., 2005; Afriyie-Gyawu et al., 2008a; Afriyie-Gyawu et al., 2008b; Phillips et al., 2008; Wang et al., 2008). The AF exposures may be a contributing factor in the elevated incidence of hepatocellular carcinoma in these high risk populations, where detection of AF in blood and urine correlate with the frequent consumption of foods likely contaminated with AF, such as corn, corn tortillas, groundnuts, and rice (Johnson et al., 2010).

Conclusions

Exposure to AF from the diet has been associated with drought and increased risk for liver cancer, malnutrition, growth faltering, and immunosuppression. Strategies to mitigate aflatoxicosis in animals and humans are needed. Research investigating the ability of various dioctahedral smectite clays to prevent the bioavailability of AF has proven effective. Natural bentonites, consisting primarily of montmorillonite clays, are common feed additives used for this purpose. The proposed mechanism (toxin enterosorption) by which montmorillonite clay reduces the bioavailability of AF is by sequestering and "tightly" sorbing the toxin from the gastrointestinal tract onto clay surfaces, especially those within the interlayer which possesses the

highest capacity. The use of NS clay as an enterosorbent for AF is a novel strategy that is cost effective and can significantly reduce risks associated with AF exposure from contaminated diets.

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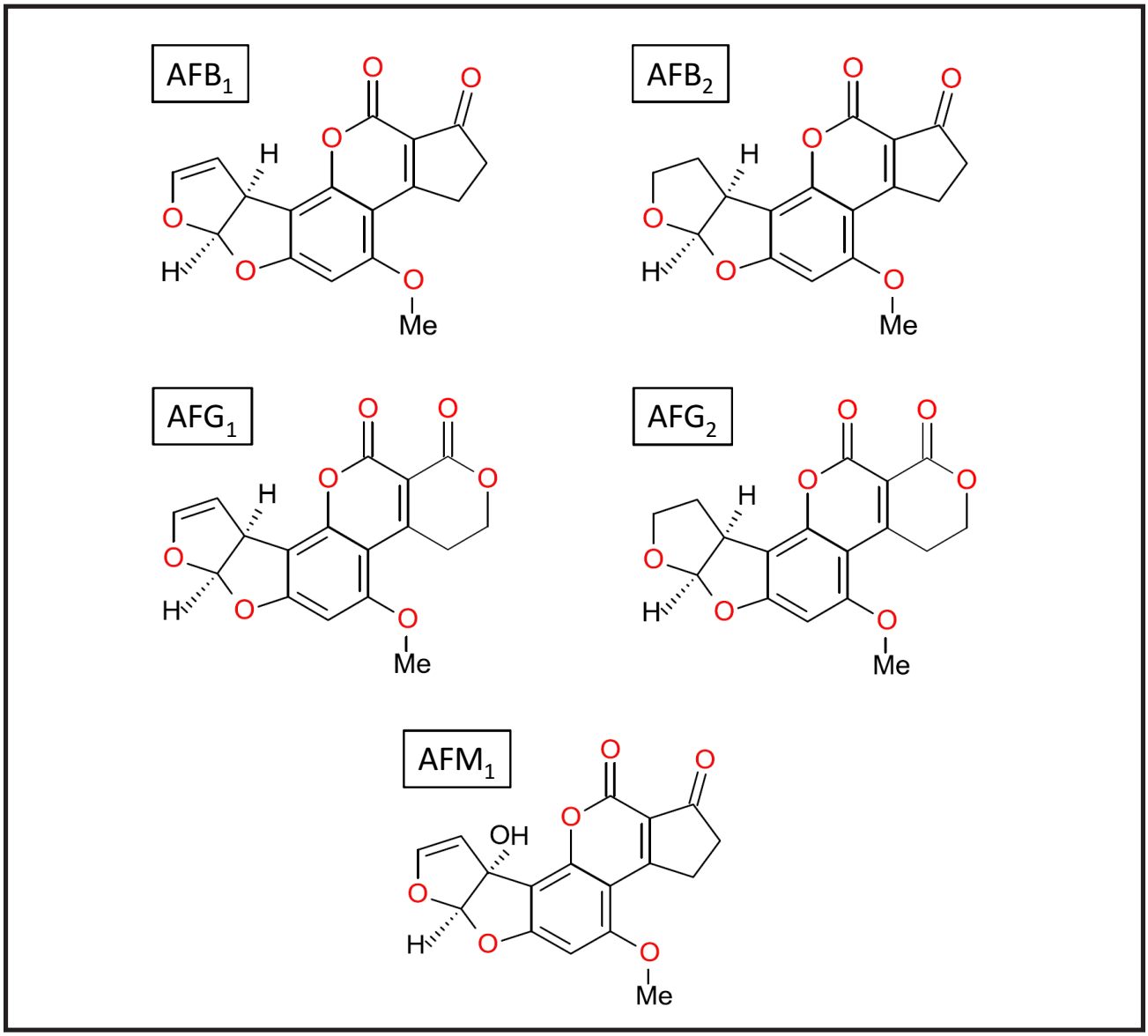

Figure 1. Molecular structures of the 4 major aflatoxin congeners (AFB_1, AFB_2, AFG_1, and AFG_2) and the important metabolic product, AFM_1.

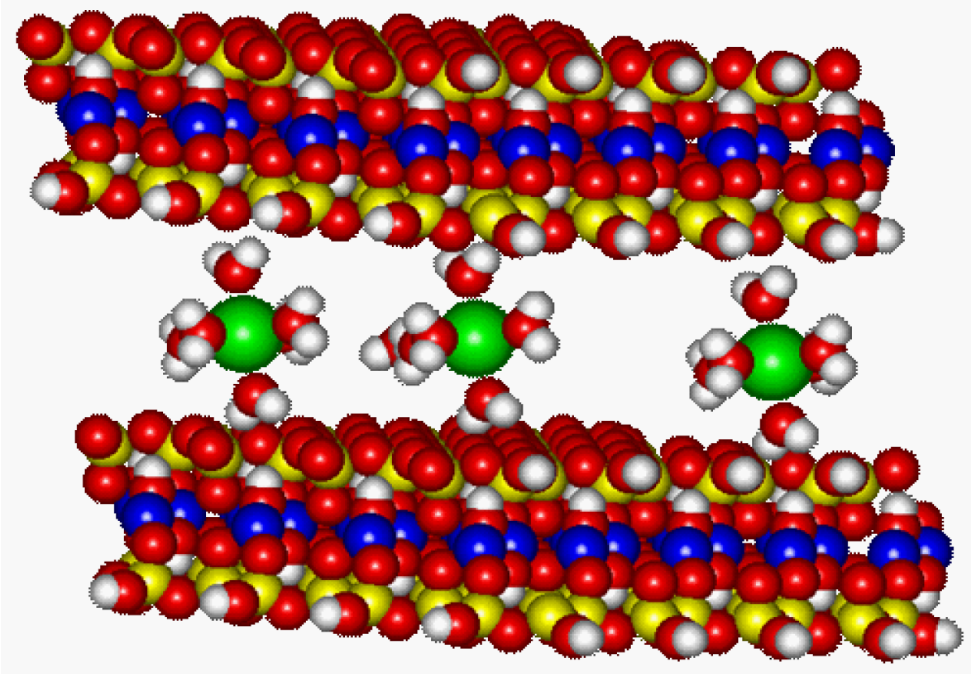

Figure 2. Schematic representation of a dioctahedral smectite clay similar to NovaSil, depicting hydrated calcium (green) as the predominant interlayer cation. Key: Si^{4+} (yellow) in the tetrahedral layer and Al^{3+} (blue) in the octahedral layer. O (red) and H (white).

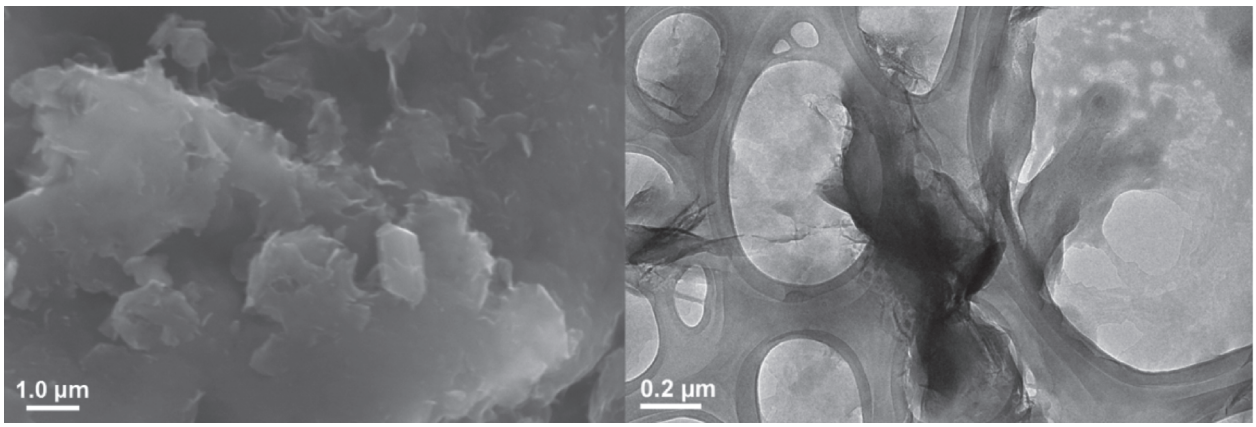

Figure 3. A) Scanning electron microspy image of a particle of NovaSil **(NS)**. B) Transmission electron microspy image of NS displaying complex morphology of sheets and rolls. This morphology is indicative of an efficient aflatoxin adsorbent (Mulder et al., 2008).

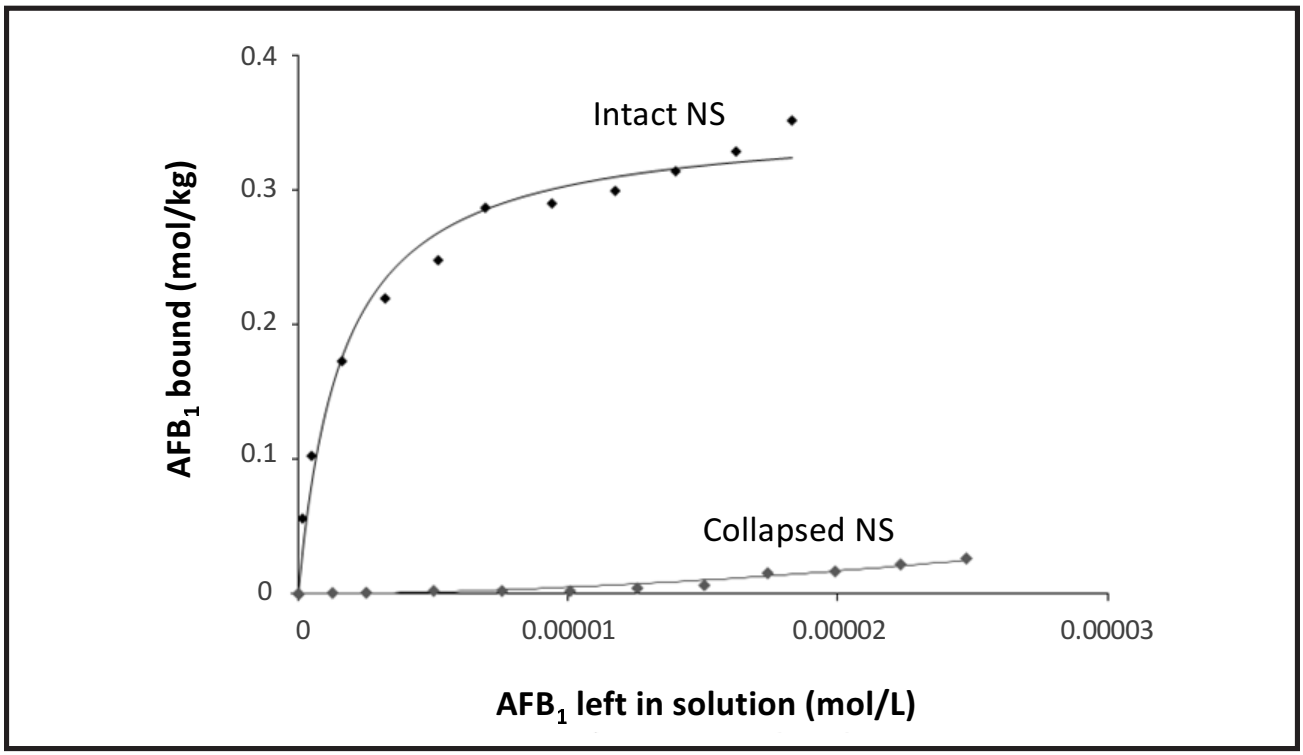

Figure 4. Aflatoxin adsorption isotherms on intact and collapsed NovaSil (NS) clay (ca-montmorillonite). Collapse was achieved after heating the clay at 800°C for 1 h.

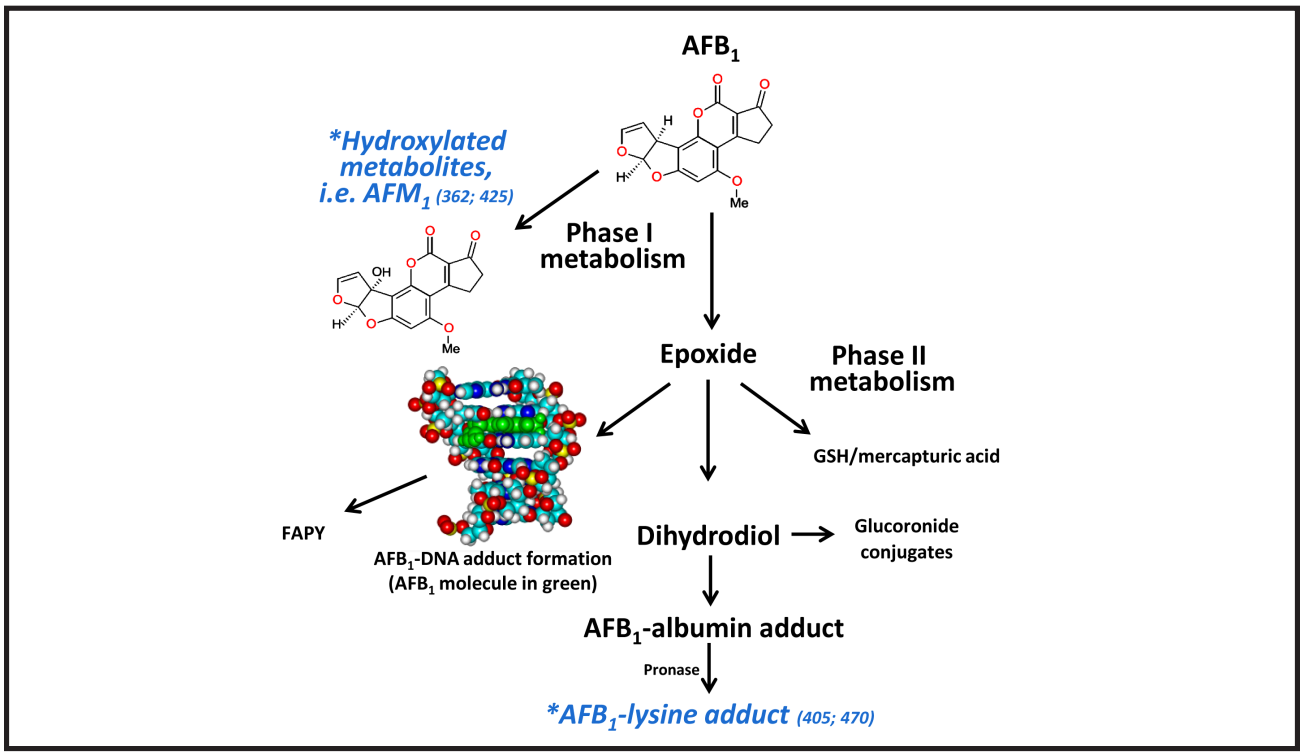

Figure 5. The AFB_1 metabolism produces a series of potential exposure biomarkers in the blood and urine of exposed animals and humans (GSH = glutathione and FAPY = formamidopyridine). Urinary AFM_1 and the AFB_1-lysine adduct are used in our studies to monitor exposures and to evaluate aflatoxin sorbent efficacy.