

## Short-Term Safety and Efficacy of Calcium Montmorillonite Clay (UPSN) in Children

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**Abstract.** Recently, an association between childhood growth stunting and aflatoxin (AF) exposure has been identified. In Ghana, homemade nutritional supplements often consist of AF-prone commodities. In this study, children were enrolled in a clinical intervention trial to determine the safety and efficacy of Uniform Particle Size NovaSil (UPSN), a refined calcium montmorillonite known to be safe in adults. Participants ingested 0.75 or 1.5 g UPSN or 1.5 g calcium carbonate placebo per day for 14 days. Hematological and serum biochemistry parameters in the UPSN groups were not significantly different from the placebo-controlled group. Importantly, there were no adverse events attributable to UPSN treatment. A significant reduction in urinary metabolite (AFM<sub>1</sub>) was observed in the high-dose group compared with placebo. Results indicate that UPSN is safe for children at doses up to 1.5 g/day for a period of 2 weeks and can reduce exposure to AFs, resulting in increased quality and efficacy of contaminated foods.

### INTRODUCTION

Stunting, wasting, and fetal growth retardation result in more than 2 million deaths in children under the age of 5 years and account for 21% of disability-adjusted life years worldwide.<sup>1</sup> In 2010, it was estimated that 171 million preschool children worldwide were stunted.<sup>2</sup> Although the overall prevalence of stunting in developing countries has improved over the past two decades (from 44.4% to 29.2%), the majority of this improvement has been occurring in Asia and Latin America. The rate in Africa (40%), however, has remained stagnant and is not expected to improve drastically over the next 10 years.<sup>2</sup> Although stunting is primarily attributed to nutritional and protein deficiencies, aflatoxin (AF), a common maize and peanut contaminant in Africa and Asia, has also been associated with growth faltering in sub-Saharan Africa.<sup>3–10</sup> Current strategies implemented to alleviate growth faltering and subsequent physical and mental deficits include food and micronutrient supplementation. These complementary foods are designed to supplement the typical diet and often consist primarily of maize and groundnuts (peanuts), putting children at risk for AF exposure.<sup>11–13</sup> Recently, the population in which this trial was carried out was determined to be consuming homemade Weanimix with average contamination levels exceeding 200 ppb Aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) and some containing up to 500 ppb.<sup>14</sup> Because of dietary exposures in African countries, such as Guinea, Kenya, Benin, Togo, Senegal, and The Gambia, approximately 85–100% of children have detectable levels of serum or urinary AF biomarkers.<sup>3,5,7,15–19</sup>

Increasing evidence that children in sub-Saharan Africa are chronically exposed to AF has resulted in recent efforts to understand the role that AF plays in growth stunting and its influence on morbidity and mortality rates. Turner and others<sup>6</sup> followed the growth of 138 Gambian infants from birth to 12 months and compared growth status with maternal AF exposure during pregnancy. In this study, a higher mean maternal exposure level was significantly correlated

with lower weight-for-age and height-for-age *z* scores. Furthermore, children ages 16–37 months from Benin exhibited a significant negative correlation between AF exposure and height increase over 8 months.<sup>3</sup> The growth stunting observed in children from Benin and Togo had a distinct dose-response relationship with AF biomarker levels in the serum (AF-alb). Children with a height-for-age *z* score of  $\leq -2$  had 30–40% higher AF-alb compared with children not classified as stunted.<sup>4,5</sup> Aside from its effects on growth, AF is also a potent carcinogen and can have deleterious effects on immune status and liver health.<sup>7,20–24</sup> Although the exact mechanisms of AF-induced growth stunting are not well-defined at this time, animal studies have indicated that AF exposure results in decreased weight gain, lowered feed conversion efficiency, vitamin depletion, and inhibited protein synthesis.<sup>25–35</sup> As a result of these epidemiological studies, it is clear that intervention strategies directed at the mitigation of child exposures are needed in areas where risk of AF consumption is high and malnutrition is common.

Inclusion of a calcium montmorillonite clay, NovaSil (NS), in animal feed has shown efficacy in reducing bioavailability of AFB<sub>1</sub> after ingestion.<sup>36–38</sup> For example, young broiler chicks administered 5 ppm AFB<sub>1</sub> and 0.5% (wt/wt) NS clay were protected from the growth inhibitory effects of AF observed in controls.<sup>34</sup> In growing barrows, NS prevented toxicity of AF over a 4-week period, which was determined by recovery of weight gain and serum alkaline phosphatase (ALP) and  $\gamma$ -glutamyl transferase (GGT) values compared with control group levels.<sup>39,40</sup> In addition, NS has also been shown to significantly reduce AF biomarkers of exposure, including urinary metabolite (AFM<sub>1</sub>) in urine and milk and AF-alb in serum of various animal models.<sup>38,41–43</sup> Human intervention trials with NS products in Texas and Ghana have also reported a similar reduction of AF bioavailability, resulting in as much as a 58% decrease in biomarker levels.<sup>44,45</sup>

Recently, the NS parent compound was refined, producing a product that is more uniform in particle size, with the majority of the particles residing in the 45- to 100- $\mu$ m range. The new product, Uniform Particle Size NovaSil (UPSN), displayed a similar AFB<sub>1</sub> sorption capacity to that of NS during isothermal analysis and was deemed to be more palatable for human consumption.<sup>46</sup> *In vivo* efficacy of UPSN was verified in Ghanaian adults during a crossover-designed

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trial, in which UPSN was added to participants' food before ingestion.<sup>45</sup> Results from mineralogical analyses of UPSN and NS indicated similar structural, morphological, and chemical characteristics; therefore, the two materials are thought to possess similar safety profiles.<sup>46</sup>

Safety assessment of both NS and UPSN has been conducted in animal and adult human trials.<sup>36,46-48</sup> Results of NS clay phase I and II clinical trials suggest that ingestion of up to 3 g/day in adults is safe for a 3-month period.<sup>49-51</sup> Based on detailed studies conducted in animals and humans, it was determined that ingestion of UPSN at levels efficacious for reducing AFB<sub>1</sub> biomarkers would be reasonably safe in children.

In this clinical trial, safety and efficacy of UPSN were assessed for children at risk for AF exposure from the Ejura-Sykedumase district of Ghana. The study followed a double-blind, placebo-controlled trial design over a 2-week time period. The results from this research will be used to design future studies investigating long-term protection of children at high risk for AF exposure and the potential of this material for short-term therapy during outbreaks of acute aflatoxicosis.

## MATERIALS AND METHODS

**Materials.** UPSN was obtained from BASF (Jackson, MS). UPSN was examined for various environmental contaminants, including dioxins and heavy metals, to ensure compliance with federal and international standards. Metal and dioxin analyses of both NS (BASF) and UPSN were reported to be similar and well under the tolerable daily intake (TDI) or provisional tolerable daily intake (PTDI) set forth by the World Health Organization (WHO) and the Joint Food and Agriculture Organization/WHO Expert Committee on Food Additives (JECFA).<sup>46</sup> UPSN was sterilized by electron beam irradiation to prevent any possible bacterial or viral contamination before trial initiation.

High-performance liquid chromatography-grade methanol, phosphate-buffered saline, and AFM<sub>1</sub> standard were purchased from Sigma Aldrich (Saint Louis, MO). Ultrapure deionized water (18.2 MΩ) was generated within the laboratory using an Elga Automated Filtration System (Woodridge, IL). Immunoaffinity columns were purchased from VICAM (Milford, MA).

**Study site and participants.** Study participants were recruited from six communities in the Ejura-Sekyedumase district of the Ashanti Region of Ghana. The six communities included Dromonkuma, Hiwoanwu, Kotokoliline, Nkwanta, Ejurafie, and Kasei. These communities are in rural areas, where inhabitants are primarily subsistence farmers. All recruited participants were between 3 and 9 years of age. Consent was sought from the parents or legal guardians after a community meeting with study personnel. Consent documents were translated and explained to each participant, and then signed by each participant's guardian before initiation of the study. Participants were randomly assigned to one of three treatment groups. The three treatment arms consisted of a placebo group, which received 0.75 g calcium carbonate two times daily, a low-dose group, which received 0.375 g UPSN two times daily, and a high-dose group, which received 0.75 g UPSN two times daily. A placebo-controlled group was deemed necessary in this research because clinical reference ranges for hematology and serum biochemistry values are not currently well-established for African children.<sup>52</sup> Thus,

the placebo group was used as a reference when determining safety of UPSN. Doses were weighed into identical packages at the Noguchi Memorial Institute for Medical Research (NMIMR) before the study to ensure that monitors and participants would be blinded to their treatment. Trained study monitors mixed each participant's treatment into their normal breakfast and dinner meals provided by the participant's parents. No additional foods were provided by the clinical team. Breakfast meals typically consisted of a corn-based porridge called koko or soup, and the dinner meals typically consisted of a common soup (i.e., peanut soup or lamb lite soup) and corn or cassava dough called banku and fufu, respectively. These meals were supplied by the individual households. Participants provided blood samples (3 mL) on the morning before initiation of treatment (day 0) and day 15 (the morning after their last treatment dose). Blood samples were collected by trained phlebotomists at the Ejura District Hospital. Aliquots of the blood samples were used for hematological analysis, and the remaining amount was centrifuged. The resulting serum was collected and kept at -20°C. Urine samples were collected by parents on the morning of day 0 (baseline), halfway through the study (day 7), and the morning after the final dose (day 15). After collection, urine samples were stored at -20°C and together with the serum samples, transported to NMIMR for analysis. The study design followed the guidelines for a double-blind randomized clinical trial. Ethical clearance and Institutional Review Board approval for this study were obtained from both Texas A&M University and NMIMR (2011-0684 and 043/11-12, respectively).

**Adverse events monitoring.** Based on the existing scientific literature describing consumption of dioctahedral smectite clays in adults and children, no severe toxicity was expected as a result of UPSN treatment. However, research staff and medical personnel were on site throughout the study period to monitor for potential adverse effects and remove participants from the study in the event of such an effect. Daily diary worksheets and symptom checklists were provided to study monitors as assessment tools for adverse events monitoring and completed two times daily after ingestion of each treatment dose. Adverse events are described as percentages of the total numbers of adverse event reports out of the total numbers of completed daily diary worksheets per treatment group. In the event of an adverse treatment effect or unrelated condition at any time during the study, medical treatment was available to participants from the district hospital at no cost to the participant. Any symptoms were assessed according to the following criteria: mild (grade 1), slightly bothersome and relieved with symptomatic treatment; moderate (grade 2), bothersome and interfered with activities and only partially relieved with symptomatic treatment; severe (grade 3), prevented regular activities and not relieved with symptomatic treatment. Any participant experiencing a severe symptom was advised to seek immediate medical attention. Physical examination and laboratory analysis were performed for persistent symptoms. Any symptoms that were linked to the UPSN treatment by the study physician would result in immediate discontinuation of the treatment; however, this did not occur during the study.

**Hematology and serum mineral analysis.** Whole-blood measurements consisted of hemoglobin, total white cell count, and platelet count. Whole-blood analysis was conducted with

a flow cytometer (Abx micro60; Block Scientific, Bohemia, NY). Serum albumin, alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGT), total protein, total bilirubin, urea, creatinine, triglycerides, sodium (Na<sup>+</sup>), potassium (K<sup>+</sup>), chloride (Cl<sup>-</sup>), calcium (Ca<sup>2+</sup>), and magnesium (Mg<sup>2+</sup>) were measured using a Flexor E automatic blood analyzer (Vital Scientific, Dieren, Netherlands).

**AFM<sub>1</sub> analysis.** Analysis of urinary AFM<sub>1</sub> levels followed previously published methods<sup>38,53</sup> that have been validated by our laboratory.<sup>44</sup> After immunoaffinity column (Aflatest WB; VICAM) clean up, urine samples were analyzed using a high-pressure liquid chromatography (HPLC) system with fluorescence detection capability (Shimadzu Corp., Kyoto, Japan). Urinary AFM<sub>1</sub> concentrations were expressed as picograms per milligram creatinine to correct for variations in urine dilution among samples. Creatinine concentrations were measured by a Flexor E Autoanalyzer.

**Statistical analysis.** Statistical analysis was conducted with JMP 10 software (SAS Institute, NC). The ultimate goal of this study was to determine if the ingestion of UPSN clay was safe in children; therefore, statistical evaluation focused on comparisons between treatment arms as well as values within a group at baseline and day 15. A  $\chi^2$  test was used for analysis of side effect/toxicity data between treatment groups. As expected, the AFM<sub>1</sub> biomarker of exposure data was not normally distributed and therefore, was log-transformed before analysis. Paired *t* test and analysis of variance (ANOVA) statistical tests were conducted on both AFM<sub>1</sub> data and biochemical parameters for comparisons among treatment groups. A two-tailed *P* value < 0.05 was considered statistically significant. Correlation analyses were performed for serum biochemical parameters and AFM<sub>1</sub> levels. *P* values and correlation coefficients were calculated by a Pearson correlation test to evaluate the association between bilirubin and AFM<sub>1</sub> levels.

RESULTS

**Study participant characteristics and compliance.** In total, 63 child participants were enrolled in the clinical trial. There were no significant differences in mean age, sex, weight, or other general physical parameters, such as blood pressure, between treatment groups (Table 1). Also, there was no

TABLE 1  
Demographics and physical parameters

Demographic characteristics	Treatment group		
	Placebo	Low dose	High dose
Participants	21	21	21
Sex			
Male	13	14	14
Female	8	7	7
Age (years)*	5.8 ± 1.6	5.4 ± 1.8	5.7 ± 1.9
Body weight (kg)*	20.0 ± 4.8	21.0 ± 8.5	18.9 ± 5.2
Height (cm)*	107.2 ± 13.2	107.9 ± 16.3	105.8 ± 15.1
Height-for-age z score	-1.5 ± 1.9	-0.8 ± 1.8	-1.4 ± 1.6
Weight-for-age z score	-0.4 ± 1.5	0.2 ± 1.0	-0.6 ± 1.2
BMI (kg/m <sup>2</sup> )	17.3 ± 1.8	17.7 ± 3.3	16.7 ± 1.7
Systolic blood pressure (mmHg)*	87.7 ± 8.0	91.3 ± 10.9	92.3 ± 11.0
Diastolic blood pressure (mmHg)*	44.6 ± 7.3	45.6 ± 9.1	47.2 ± 10.2

Note that all data are baseline values.  
\*Mean ± SD.

trend in the calculated body mass index (BMI) values for participants versus the percentage reduction of AFM<sub>1</sub> levels in urine. Although the average height-for-age z score was -1.2 ± 1.8, values ranged from -6.01 to 2.14, indicating that nutritional status varied significantly in the population. Adherence to the 2-week study protocol was excellent, with all 63 participants completing the study. Only one participant missed an evening dose of treatment throughout the 28 doses per participant administered. This participant was in the low-dose UPSN group and diagnosed with and treated for malaria that same day. General acceptance by the parents and children was exceptional.

**Adverse events and side effects.** The two dose levels of UPSN (0.75 and 1.5 g/day) were tolerable to the participants throughout the study. Adverse symptoms reported during the 2-week study were primarily of a gastrointestinal nature and included vomiting and diarrhea. Table 2 is a tally of individual adverse events reported throughout the course of the study. It is important to note that, in some cases, reports were made multiple times by the same participant. For example, all reported events in the high-dose group originated from one individual who received medical attention from the district hospital after 2 consecutive days of vomiting. This participant was diagnosed and treated for malaria after visiting the hospital but allowed to stay in the clinical trial per the physician's recommendation. Vomiting ceased after initiation of malarial medication. In total, there were three children diagnosed and treated for malaria during the study. This is equivalent to 4.8% of the study population, which is actually a lower prevalence rate than reported for the area (> 75 per 1,000) by the WHO.<sup>54</sup> The symptoms reported by individuals with malaria accounted for 9 of the total 13 adverse events (69.2%) reported. The placebo, low-dose, and high-dose groups experienced side effects at rates of 0.3% (2 of 588), 0.8% (5 of 588), and 1% (6 of 588), respectively. Severities of side effects were generally reported as mild to moderate, and either no treatment or self-treatment was effective in alleviating symptoms. Severe cases of vomiting requiring immediate medical attention occurred only in those

TABLE 2  
Adverse events reported

Symptom reported	Treatment group			Sum
	Placebo	Low dose	High dose	
Other	0*	0	2†	2
Indigestion	0	0	0	0
Nausea	0	0	0	0
Vomiting	2	3†	4†	9
Constipation	0	0	0	0
Diarrhea	0	1	0	1
Flatulence	0	0	0	0
Loss of appetite	0	1	0	1
Abdominal discomfort	0	0	0	0
Heartburn	0	0	0	0
Dizziness	0	0	0	0
Insomnia	0	0	0	0
Bloating	0	0	0	0
Total incidence (%)	2 (0.3)	5 (0.8)	6 (1)	13 (0.7)
Severity				
Mild	2	2	5	9
Moderate	0	2	1	3
Severe	0	1	0	1

\*Indicates number of times an adverse event was reported.  
†Indicates that the participant was diagnosed with malaria by health officials.

TABLE 3  
Hematological analysis

	Treatment group						Normal clinical range (United States)	Reference
	Placebo		Low dose		High dose			
	Before	After	Before	After	Before	After		
Hemoglobin (g/dL)	11.3 ± 0.8	11.4 ± 1.1	11.5 ± 0.9	11.7 ± 1.3	11.0 ± 1.5	11.3 ± 1.5	11.0–14.5	A
WBC (×1,000/mm <sup>3</sup> )	8.2 ± 2.0	7.4 ± 1.2	7.8 ± 2.0	7.9 ± 1.8	8.5 ± 3.4	8.2 ± 2.7	3.4–12.0	A
Platelet (×1,000/mm <sup>3</sup> )	275.3 ± 73.9	303.0 ± 112.4	296.0 ± 85.0	349.9 ± 142.7	266.0 ± 80.4	280.9 ± 117.3	150.0–450.0	A
Lymphocytes (%)	55.2 ± 10.9	56.9 ± 8.1	52.6 ± 7.9	51.8 ± 9.6	52.9 ± 11.2	53.5 ± 9.4	28.0–48.0	B
Monocytes (%)	10.6 ± 3.2	11.0 ± 3.9	12.3 ± 3.7	13.0 ± 3.9	10.4 ± 3.0	10.9 ± 3.3	3.0–6.0	B
Granulocytes (%)	34.2 ± 12.1	31.9 ± 7.4	35.1 ± 8.4	35.2 ± 10.7	36.6 ± 11.0	35.6 ± 9.1	32.0–76.0	B

Data are mean ± SD. A indicates Mayo Clinic pediatric reference values. B indicates Children's Hospitals and Clinics of Minnesota reference values. Reference ranges are combined for males and females. WBC = white blood cell.

participants later diagnosed with malaria. Importantly, there were no significant differences observed in the numbers of adverse events between treatment groups ( $P = 0.37$ ) or the severity of symptoms reported ( $P = 0.43$ ).

#### Hematological, blood chemistry, and serum mineral effects.

Hematological analysis of blood samples indicated no significant difference between treatment arms, although lymphocytes and monocytes were above the normal range across all treatment groups at both days 0 and 15 (Table 3). Hematological values in the placebo group were not significantly different from either the low- or high-dose UPSN groups at day 0 or 15. These levels were also not significantly different within each treatment group comparing day 15 values with baseline values.

Results of serum biochemistry analyses are provided in Table 4. No significant differences were observed within groups between days 0 and 15 for albumin, ALP, AST, GGT, total bilirubin, urea, or triglycerides. ALT values were significantly higher after treatment in all groups ( $P < 0.0003$ ), including the placebo group. The placebo group also exhibited significantly lower ( $P < 0.01$ ) total protein levels after treatment; however, this was not the case for the UPSN-treated groups. At day 15, the low-dose UPSN group had significantly lower creatinine levels compared with baseline ( $P < 0.05$ ); however, this observation was not dose-dependent. Comparison of serum biochemistry parameters between the placebo group and the low- or high-dose UPSN group did not indicate significant differences at day 0 or day 15 (Table 4). Although average total bilirubin levels were within range in all treatment groups throughout the study, a wide range of values was detected (0.2–35.5  $\mu\text{mol/L}$ ), and a weakly positive but significant ( $P = 0.005$ ) association with urinary AFM<sub>1</sub> concentration at day 0 was noted (Figure 1).

Median, mean, and normal US pediatric ranges for the serum minerals assessed are outlined in Table 5. Serum Na<sup>+</sup> and Cl<sup>-</sup> significantly increased ( $P < 0.01$ ) over the course of the study in all groups. All groups exhibited significantly decreased Ca<sup>+</sup> levels after the study. A reduction in serum Mg<sup>2+</sup> was seen in the UPSN high-dose group ( $P < 0.01$ ). There was also a small reduction in the mean Mg<sup>2+</sup> of the UPSN low-dose group; that was not significantly different ( $P = 0.27$ ). However, levels in all groups remained well within the normal US range. No other mineral level comparison between days 0 and 15 was significantly different.

**AFM<sub>1</sub> biomarker levels in urine.** Throughout the study, 100% of urine samples contained detectable levels of AFM<sub>1</sub>. AFM<sub>1</sub> concentration ranged from 0.5 to 5443.7 pg/mg creatinine. The mean at day 0 was 297.8 pg/mg creatinine. AFM<sub>1</sub> levels were not significantly different by sex or correlated with

age (data not shown). Although treatment groups were not significantly different at baseline or day 15 of treatment, the high-dose UPSN group exhibited significantly ( $P = 0.0216$ ) lower AFM<sub>1</sub> levels on day 7 than both the placebo and low-dose groups. When all data values from days 7 and 15 were pooled by treatment group, the high-dose group was still statistically lower ( $P = 0.0063$ ) and showed a 52% reduction in median AFM<sub>1</sub> levels compared with control (Figure 2). Pooled AFM<sub>1</sub> levels for placebo, low-dose, and high-dose groups were 549.00 (95% confidence interval [95% CI] = 416.58–681.42), 171.33 (95% CI = 150.46–192.20), and 121.87 (95% CI = 103.01–140.73), respectively.

## DISCUSSION

Chronic childhood AF exposure has gained interest over the past decade as a potential variable in the complex milieu of biological and environmental factors that lead to stunting, wasting, and suppressed immunity. In particular, sub-Saharan Africa has been identified as an area at high risk for AF exposure as well as growth stunting. Investigations from the Ejura district in the Ashanti Region of Ghana have shown ongoing, high-level AF exposure over the past decade.<sup>44,45,55,56</sup> A prevalence of up to 54.9% has been reported for stunted and/or wasted children from another district of the Ashanti Region.<sup>57</sup> Although the high rates observed in this population are primarily thought to occur as a result of inadequate nutrition and protein intake, multiple variables likely contribute to the etiology of disease. Chronic AF exposure in this community could be one such contributing factor, particularly after administration of nutritional supplements such as homemade Weanimix, which consists of groundnuts, beans, and maize (0.5:0.5:4 ratio). A recent assessment of the weaning foods produced in this community intended for children between the ages of 6 months and 2 years showed AF contamination in 100% of samples, with levels as high as 500 ppb.<sup>58</sup> Urine samples collected from children before and after 21 days of homemade Weanimix consumption revealed increased levels of urinary AFM<sub>1</sub>, indicating that, although it is an important nutritional supplement in this region, Weanimix can also cause heightened AF exposure.<sup>58</sup> Therefore, an intervention strategy to reduce childhood exposure in these populations while maintaining the use of these important nutritional supplements is of particular interest.

Enterosorption therapy may be a valuable tool in low-economic, high-risk areas, where food insecurity results in limited variety in the diet and continued consumption of poor-quality foods.<sup>37,59</sup> Clinical trials using similar dioctahedral

TABLE 4  
Serum biochemistry

	Treatment groups						Normal clinical range (United States)	Reference
	Placebo		Low dose		High dose			
	Before	After	Before	After	Before	After		
Albumin (g/L)								
49	47.2	48.5	47.9	49.6	48.4			
47.0 ± 9.0	46.9 ± 2.8	48.5 ± 3.1	47.2 ± 2.3	46.2 ± 11.1	47.6 ± 6.0	35.0–50.0	A	
15.9–56.0	39.7–51.0	42.5–53.0	42.2–51.6	11.1–57.4	25.2–56.2			
ALP (U/L)								
591.9	596.2	479	620.5	521.3	581.9			
559.1 ± 185.7	532.0 ± 355.6	427.2 ± 242.3	572.3 ± 332.6	488.5 ± 215.8	584.9 ± 314.7	149.0–468.0	A	
176.9–1,039.4	6.3–1,164.3	17.3–843.4	6.1–1,102.2	13.1–907.6	24.7–1,189.1			
ALT (U/L)								
13.2	27.1	17.2	27.6	17.7	24.6			
14.4 ± 6.0	430.2 ± 11.3*	19.3 ± 8.6	28.9 ± 7.2*	20.2 ± 20.0	29.3 ± 15.1*	7.0–55.0	A	
4.3–27.1	13.7–51.4	7.5–46.5	20.8–45.8	3.0–87.3	12.4–68.3			
AST (U/L)								
39.8	41.2	38.3	42.1	38.4	40.4			
41.8 ± 13.2	44.9 ± 12.3	42.3 ± 8.4	43.7 ± 10.8	40.2 ± 14.1	41.4 ± 10.0	8.0–60.0	A	
16.8–66.3	31.4–73.3	30.9–59.5	29.8–74.3	12.6–85.9	20.6–57.3			
GGT (U/L)								
15	16.6	10.6	14	13.2	15			
16.6 ± 8.0	17.0 ± 6.8	13.9 ± 8.6	16.0 ± 7.3	14.1 ± 9.0	17.0 ± 11.7	7.0–29.0	A	
1.7–42.0	6.1–31.4	6.4–43.2	7.4–35.5	0.5–37.2	2.8–48.7			
Total bilirubin (µmol/L)								
5.5	8.1	6.2	7.5	6.7	6.1			
7.1 ± 7.1	8.5 ± 4.9	7.8 ± 5.4	7.9 ± 3.4	8.4 ± 8.1	8.3 ± 7.4	1.7–17.1	A	
0.2–28.1	0.6–22.4	0.5–25.4	1.8–15.9	1.1–35.5	0.2–29.9			
Total protein (g/L)								
80.5	76.3	77.8	77.2	79.8	80.9			
78.8 ± 8.8	77.0 ± 3.3*	78.2 ± 6.0	77.4 ± 4.3	79.8 ± 31.0	78.0 ± 8.6	63.0–79.0	A	
43.6–87.2	70.8–82.5	70.2–89.8	70.1–86.1	21.3–194.2	47.8–89.6			
Urea (mmol/L)								
2.9	2.7	2.8	2.7	3.1	2.8			
2.7 ± 0.8	2.9 ± 0.6	2.8 ± 0.8	2.6 ± 0.6	2.9 ± 1.2	2.9 ± 1.0	2.5–7.1	A	
0.8–3.8	2.1–4.5	1.5–4.6	1.6–3.7	0.6–5.2	1.6–6.0			
Creatinine (µmol/L)								
58.1	53.3	61.7	54.3	64.4	59.1			
54.6 ± 16.4	54.3 ± 6.2	62.4 ± 10.1	55.7 ± 9.5*	60.7 ± 17.9	56.5 ± 9.0	50.0–110.0	C	
5.4–71.1	40.2–65.1	38.7–77.0	42.4–78.5	18.5–90.4	34.1–74.2			
Triglycerides (mmol/L)								
0.8	1.1	1	1	0.9	1.1			
1.0 ± 0.7	1.3 ± 0.8	1.3 ± 0.7	1.2 ± 0.7	1.6 ± 3.0	1.3 ± 0.6	< 1.02	A	
0.2–2.6	0.4–3.5	0.5–3.0	0.7–3.8	0.2–14.1	0.5–3.2			

Data represent median, mean ± SD, and range. A indicates Mayo Clinic pediatric reference ranges. C indicates Royal College of Physicians and Surgeons of Canada ranges. Reference ranges are combined male and female values.  
\*Denotes significant difference between baseline and after treatment.

smectite clays at doses as high as 6 g/day for the treatment of acute diarrhea in children resulted in limited adverse effects, of which mild constipation was the most severe event reported.<sup>60–63</sup> Similarly, results from this study indicate that administration of dietary UPSN powder at concentrations from 0.75 to 1.5 g/day in healthy children (ages 3–9 years) for 14 days resulted in minimal side effects. Neither dose-dependent toxic effects nor severe clinical symptoms were related to UPSN consumption in this study. Hematologic parameters indicated that UPSN treatment did not impair immunity or promote an inflammatory response.<sup>64</sup>

ALT values were increased in all treatment groups at day 15; however, there were no significant differences between the low- and high-dose UPSN groups and the placebo group. These values were also within normal pediatric reference ranges reported by the US Mayo Clinic (Table 4). Additionally, all other liver toxicity parameters (i.e., ALP, AST, bilirubin, and GGT) were not increased at day 15 in any treatment groups. Therefore, the cause of increased ALT values over the duration of the study remains unclear. The

placebo group experienced the greatest increase in ALT, although day 15 levels were still well within the normal range for all three groups. Sodium and chloride ion levels were also significantly increased over the study period; however, this was observed in both placebo and UPSN-treated groups, indicating little biological significance. The levels for Cl<sup>-</sup> were still within the normal reference range, whereas some Na<sup>+</sup> values were out of range based on the US pediatric values, which is shown in Table 5.<sup>65</sup>

Increases in Ca<sup>2+</sup> have been observed after administration of the parent NS product and UPSN in rats, which was attributed to dissolution of calcite and exchangeable Ca<sup>2+</sup> ions from montmorillonite.<sup>46,47</sup> However, in this study, total Ca<sup>2+</sup> levels were decreased in all treatment groups, including the placebo (calcium carbonate). Calcium carbonate and, to a lesser extent, NS clay typically act as calcium supplements; thus, it is likely that this overall reduction in serum levels is a result of dietary changes during the intervention trial.

Magnesium ion was the only serum micronutrient that dose-dependently decreased with UPSN treatment. The mean

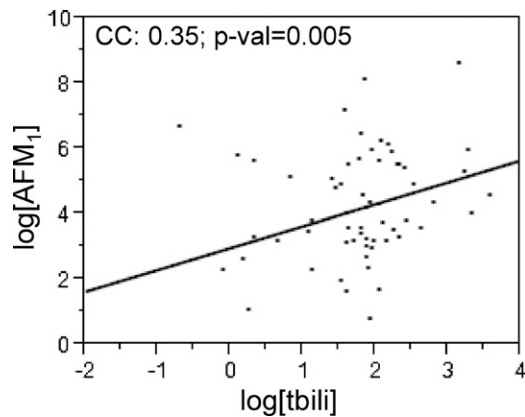


FIGURE 1. Correlation of log-transformed total bilirubin and log-transformed AFM<sub>1</sub> values for all participants at day 0.

concentrations in the high-dose group were significantly lower than in the placebo group on day 15 after treatment, and the low-dose group, although not significantly different, showed a decreasing trend from the placebo. However, it is important to note that all levels remained within the normal range throughout the study. Furthermore, significant modulation in serum Mg<sup>2+</sup> concentrations has not been observed in any other animal or human study with UPSN or parent NS. However, decreased absorption and retention of Mg<sup>2+</sup> were observed in a pig model after ingestion of 1% sodium montmorillonite clay.<sup>66</sup> Magnesium levels are controlled by the kidneys and gastrointestinal tract and seem to be closely linked to calcium, potassium, and sodium metabolism.<sup>67</sup> Therefore, the change in Mg<sup>2+</sup> observed here could have resulted from changes in calcium or sodium metabolism and not directly from UPSN treatment. An alternative explanation for the lowered serum Mg<sup>2+</sup> observed is a direct sequestration of Mg<sup>2+</sup> by UPSN in the gut through cation exchange activity of the clay, thus reducing the availability of Mg<sup>2+</sup> for absorption from the gut.

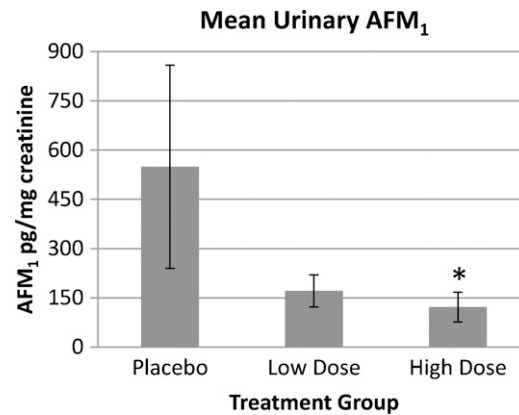


FIGURE 2. Comparison of urinary AFM<sub>1</sub> levels for placebo and low- and high-dose treatment groups. AFM<sub>1</sub> data for each group were pooled for days 7 and 15. \*Indicates statistical significance ( $P < 0.05$ ).

Longer safety trials controlling for intake of essential dietary nutrients are warranted to determine whether UPSN could interfere with micronutrient or mineral absorption in children.

Although changes in serum bilirubin have been reported after exposure to AF in animal species, to our knowledge, there have been no correlations made between AF exposure and bilirubin levels in humans.<sup>68,69</sup> The AFB<sub>1</sub>-albumin adduct, although a valuable AF assessment tool in the serum, is a long-term biomarker of exposure and not known to fluctuate with recent exposure as rapidly as the urinary biomarker. For this reason, urinary AFM<sub>1</sub> is a more appropriate marker to correlate with dynamic serum components, such as bilirubin. As stated previously, AF has been shown to elevate total bilirubin and ALP levels in animal models. Because growth stunting has been reported to be common in most forms of chronic liver disease, it will be important to assess liver function parameters in future studies involving children, growth stunting, and AFs.<sup>70</sup> Additionally, because direct

TABLE 5  
Serum mineral analysis

	Treatment groups						Normal clinical range (United States)	Reference
	Placebo		Low dose		High dose			
	Before	After	Before	After	Before	After		
Na (mmol/L)								
	132.2	138.2	135.3	138.1	128.5	135.6		
	130.6 ± 6.8	139.2 ± 5.5*	133.6 ± 8.0	138.8 ± 3.8*	129.3 ± 9.1	136.8 ± 3.1*	135.0–145.0	A
	115.3–141.4	132.2–153.4	114.3–146.2	134.5–150.3	112.1–149.9	132.7–143.3		
K (mmol/L)								
	3.7	4	3.8	3.9	3.7	4.1		
	3.8 ± 0.4	4.0 ± 0.4	3.8 ± 0.4	4.1 ± 0.5	3.8 ± 0.5	4.1 ± 0.4	3.6–5.2	A
	3.0–4.8	3.3–5.0	2.7–4.5	3.2–5.7	3.1–4.8	3.3–4.7		
Cl (mmol/L)								
	97.3	101.2	98.8	102.4	96.2	100.4		
	97.4 ± 4.2	101.2 ± 4.0*	97.7 ± 5.6	101.5 ± 2.5*	96.6 ± 5.1	100.3 ± 2.1*	102.0–112.0	A
	89.4–105.3	90.1–108.4	83.0–106.8	96.0–104.8	87.8–106.3	95.4–105.2		
Ca (mmol/L)								
	2.4	2.1	2.3	2.2	2.3	2.1		
	2.4 ± 0.2	2.2 ± 0.3*	2.3 ± 0.1	2.2 ± 0.2*	2.3 ± 0.2	2.1 ± 0.3*	2.4–2.7	A
	1.9–2.7	1.8–2.8	2.0–2.6	1.2–2.6	2.0–2.6	1.5–2.5		
Mg (mmol/L)								
	0.9	0.8	0.9	0.8	0.8	0.8		
	0.8 ± 0.1	0.8 ± 0.1	0.9 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1*	0.7–1.0	A
	0.5–1.0	0.6–1.0	0.7–1.0	0.6–1.0	0.7–1.1	0.6–0.9		

Data represent median, mean ± SD, and range. A indicates Mayo Clinic pediatric reference ranges. Reference ranges are combined male and female values.  
\*Denotes statistical significance between baseline and after treatment.

bilirubin can be measured in the urine of individuals experiencing liver malfunction, it may be an excellent non-invasive biomarker to monitor in clinical AF studies.

Although the range of AFM<sub>1</sub> excretion was similar, the average levels were significantly lower in this study than those seen in adults from the same population in October of 2010.<sup>45</sup> This finding may be explained by the fact that this intervention trial was carried out during the wet season, whereas the adult study took place at the beginning of the dry season, which typically correlates with increasing AF exposure.<sup>16,71</sup> Also, the variance in excretion levels could be attributed to the difference in food intake, metabolism, and urinary output between children and adults. The high-dose UPSN group showed a significant decrease in AFM<sub>1</sub> excretion compared with the placebo group (52%). This decrease in AFM<sub>1</sub> is similar to the percentages previously reported after NS and UPSN consumption in adults (45%, 55%, and 58.7%).<sup>44,45</sup>

The results from this clinical intervention study indicate that UPSN consumption by children (ages 3–9 years) is safe at a dose up to 1.5 g/day for 2 weeks. Inclusion of UPSN in weaning foods could also significantly decrease the amount of bioavailable AFB<sub>1</sub> from contaminated diets, thereby reducing adverse effects of AF exposure and enhancing the quality, efficiency, and safety of nutritional supplements.

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