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## THE BIOLOGICAL CONSEQUENCES OF KAOLIN GEOPHAGIA

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### Abstract

Kaolin geophagia is associated with the relief of gastrointestinal distress, but it may also cause adverse health effects on the body. This study was designed to: assess kaolin composition; test if 129SvEv mice would consume kaolin and determine the consequences of consumption; and assess rotational stress modulation of consumption. Thirteen kaolin samples were purchased from Alabama and Georgia stores. Chemical and physical properties were characterized for each sample using a Munsell chart, pH meter, Energy Dispersive Spectroscopy, Transmission Electron Microscopy, Visible Near-Infrared Spectra, and Inductively Coupled Plasma-Optical Emission Spectrometry. Kaolin was then given to mice as food supplements and consumption was determined by weight/volume consumed and recorded in 12 hour intervals. Soil physical/chemical, mouse hematological, blood chemical and kaolin consumption data suggested that kaolin contained various elements, and geophagia was not exacerbated by rotational stress.

**Keywords:** Kaolin, Pica, Geophagia, Hematology, Composition

### Introduction

Pica is the consumption of non-nutritive substances, such as charcoal, grass, and plaster (Liu et al., 2006; Mitchell et al., 1977). Hypotheses to explain the physiological causes of pica include hunger, micronutrient deficiency, toxins/pathogens, and gastrointestinal malaise, (Mitchell et al., 1977; Young, 2008). Pica may also cause nutritional deficiencies (Mitchell et al., 1977). A form of pica is geophagia (De Jonghe et al., 2009), which is the intentional consumption of soil, (Diamond, 1999; Wilson, 2003). Complications from geophagia include hyperkalemia, hypogonadism, and iron deficiency (Halstead, 1968). Accidental geophagia occurs when people eat inappropriately washed vegetables and may cause health problems if the soil contains pathogens (Parry-Jones and Parry-Jones, 1992; Pennisi, 2008).

Geophagia is often reported in pregnant women and children (Callahan, 2003; Grigsby, 2004), and may provide important minerals, like calcium, needed for the development of the fetus (Lopez-Jaramillo, 2005; Whitlin and Sabai, 1997). Soils contain high levels of minerals and trace elements (Johns and Duquette, 1991; Hunter 2008), but the bioavailability of these chemicals is not well characterized. Trace elements, substances which are important for metabolism, occur in soils in small quantities, but when present in excessive amounts, may be toxic to living systems (Adriano, 1986; Miller and Gardiner, 2001; USGS, 1974). Trace elements include heavy metals (Cd, Cr, Co, Cu and Ni); micronutrients (Cu, Fe, Mn, Mo, and Zn) and metalloids (As). In the southeastern US, “White Dirt” a type of kaolin clay can be purchased in local stores and is a common soil source for geophagous customers. Kaolin, also known as aluminum silicate, is type of clay that is normally used to produce cosmetics, medicines, ceramics, and paper. Kaolin consumption can be classified as a type of pica, can lead to health risks, and is associated with iron deficiency (Garnier et al., 2008). Biologically, kaolin can exchange and absorb cations, such

as iron, which could lead to iron-deficiency anemia (Coltman, 1969). Other data suggest kaolin consumption protects animals from illness and enhances recovery (Vera et al., 2006).

Information concerning the clinical consequences of kaolin geophagia is scant, although kaolin geophagia has been documented in patients (De Jonghe et al., 2009). Kaolin geophagia has been associated with the elimination of food allergies as well as adverse health effects, like inflammation, and infection. (Wilson, 2003, Liu et al., 2006). This study was designed to assess kaolin composition; test if 129SvEv mice would consume kaolin and determine the consequences of consumption; and assess rotational stress modulation of consumption.

## **Methodology**

### **Kaolin Samples**

Thirteen samples of kaolin were purchased from grocery stores and local gas stations in Alabama and Georgia. Samples originated from local distributors who dug the kaolin clay up from local mineral deposits using industrial (i.e., backhoe) equipment. The soil was allowed to air-dry, broken or collected as moderately-sized chunks and placed into plastic sandwich bags. Bags had photocopied labels that were stapled on them. The soil samples were not processed in any way that altered their physical characteristics, chemical properties, or composition. Distributors delivered the samples to each store in cardboard boxes with 50 bags of soil/box.

### **Physical and Chemical Determination**

The hue (color), value (lightness or darkness), and chroma (color saturation or brilliance), of all 13 kaolin samples, were determined using the Munsell Soil Color Chart (Munsell, 2004). To each kaolin sample, 0.1ml of water was added and the Munsell color determination of the wet sample was done by comparison of sample colors with the Munsell Chart. Each comparison was carried out by 3 different lab members, to ensure accuracy, and objectivity.

The pH of each kaolin sample was determined using a 1:2 kaolin-to-water ratio. Ten grams of each sample was mixed with 20mL of deionized water to produce a slurry. The resulting slurry was allowed to sit for ten minutes and pH was measured using a S500 pH meter (Daigger & Co., Vernon Hills, Illinois USA). The probe was rinsed with deionized water between readings.

### **Microscopic Analysis**

Transmission Electron Microscopic (TEM) analysis was performed on as-received white dirt clay powder using a JEOL-2010 TEM (JEOL USA, Peabody, MA). Energy Dispersive Spectroscopy (EDS) was also carried out using an Oxford EDS (Oxford Instruments, Tubney Woods, Abingdon, Oxfordshire, UK) spectrometer attached to the JEOL 2010 TEM. The white dirt clay powder samples for TEM were prepared by ultrasonic dispersion of powder particles in ethanol. A drop of the resulting solution was placed on a carbon-coated copper grid (copper grid-200 mesh), air-dried, then used for TEM analysis. A second white dirt clay powder sample was also prepared by ultrasonic dispersion of powder particles in ethanol and a drop of the solution was placed on a Molybdenum grid (grid-200 mesh), air-dried, and used for TEM analysis.

### **Microwave Digestion (MARS 5) and Elemental Analysis**

All chemical reagents used were of trace metal grade and were procured from Thomas Scientific, Inc. (Swedesboro, NJ USA). Prior to digestion, all glassware used in this study were pre-soaked

overnight in a dilute (1:11) trace metal grade nitric acid (HNO<sub>3</sub>) bath, washed, rinsed repeatedly with deionized water, and air dried. A 0.5 g (<100 mesh) aliquot of each soil sample was weighed and placed in a 75 ml Teflon microwave digestion vessel; nine milliliters of concentrated nitric acid (HNO<sub>3</sub>) was added and allowed to sit for 15 minutes. Samples were then mixed thoroughly and placed in the carousel of a Microwave Accelerated Reaction System (MARS Xpress, CEM Corporation, Matthews, NC) and digested according to the EPA 3052 Method. Following digestion, each sample was allowed to cool down, transferred into a 50-mL volumetric flask and brought to volume with deionized water. The sample was mixed and filtered through a 0.45- $\mu$ m membrane filter (Osmonics, Inc., Minnetonka, Minnesota) into a clean polyethylene bottle and analyzed using an inductively coupled plasma atomic emission spectrometer (Thermo Elemental IRIS II XSP, Franklin, MA, USA) for total macronutrient (non-trace-Ca, K, Mg, P, S, and Mn) and micronutrient (trace-As, Cd, Mo, Pb, Se, Zn, B, Na, Cu, Co, Cr, Ba, and Ni) element concentrations at Brigham Young University's Plants and Soils Laboratory in Provo, Utah. Sample blanks and control standards were used to ensure accuracy.

### **Mice**

Eight breeding pairs of mice (*Mus musculus*) strain 129S6/SvEeV, IL-10<sup>-/-</sup>, were received from the Gnotobiotic Unit of the Mutant Mouse Resource Center, University of North Carolina, Chapel Hill, NC (IACUC approval number R076-10-2). Mice were housed under specific pathogen free conditions in the Comparative Medicine Resource Center, Tuskegee University and bred via brother:sister mating to produce the mice used in this study. Mice were placed in sterile polycarbonate cages, on sterile hardwood Beta-Chip bedding (Northeastern Products, Warrenburg, NY) and were offered irradiated rodent chow (Purina Laboratory Chow, 5001, Ralston Purina Co., St. Louis, MO) and sterile (autoclaved) drinking water *ad libitum*. Bedding, water bottles, and cages were changed each week and the cage racks were changed every two weeks. The room temperature and relative humidity were maintained at 68°F±2°F and 50%±10%, respectively. Mice were routinely examined by the attending veterinarian and the CMRC staff. None of the mice were found to be injured or diseased at the initiation of this study.

### **Kaolin Consumption Study**

A total of 48 mice were randomly assigned to four different groups; “No Kaolin” (control group), “All, Kaolin”, “Kaolin 1:1”, and “Kaolin 1:1R (R=Rotational).” There were 12 mice in each group. Approximately, 130-195g of Grandma's Own Kaolin was added to each cage of mice. This brand of kaolin was used because it was purchased in the greatest supply. Standard rodent chow was added to “No kaolin” (controls) and Kaolin1:1 cages. Consumption of kaolin and chow were measured and recorded each day. Each of the 3 stationary treatment groups contained 6 male and 6 female mice. The female mice were nulliparous. The rotational group, Kaolin 1:1R, contained 12 female mice only. Mice in the rotational group were spun fifty times in a bingo ball-style cage at 12 hour intervals. Mice were then placed back in their cages and given access to kaolin, water, and chow. Behavioral observations of the mice in each group were also recorded. At the end of the experiment all mice were euthanized via CO<sub>2</sub> asphyxiation. Cardiac punctures were used to collect blood and the entire GI tracts were excised and stored.

### **Blood Chemistry**

Whole blood samples were taken from the mice in each of the four groups, pooled by group, and placed in serum collection tubes. The blood samples were then subjected to liver, kidney and

electrolyte panels using an ACE-ALERA Chemistry Analyzer (Alfa-Wasserman, Inc, West Caldwell, NJ). The kidney panel assessed levels of Phosphorus, Carbon Dioxide, Calcium, Blood Urea Nitrogen, Creatinine, Sodium Total Protein, Chloride and Albumin. The Liver Panel assessed levels of Creatine Kinase, Alanine AminoTransferase, Aspartate Transferase, Total-Direct Bilirubin, Total Protein, Albumin, Amylase, Glucose, Triglycerides, and Cholesterol.

### **Electrolytes and Hematology**

Whole blood collected from mice was added to EDTA tubes, pooled by group, and subjected to an analysis of Sodium, Chloride, and Carbon Dioxide. The ACE-ALERA Chemistry Analyzer (Alfa-Wasserman, Inc, West Caldwell, NJ) was used for these analyses. Whole blood collected from mice in each treatment group was added to EDTA tubes, pooled by group, and subjected to a Complete Blood Cell (CBC) analysis using a CELL\_DYN 3700 (Abbott Laboratories, Abbott Park, Illinois). The CBC measured the levels of White Blood Cells, Neutrophils, Lymphocytes, Monocytes, Eosinophils, Basophils, Red Blood Cells, Hemoglobin, Hematocrit, Mean Cell Volume, Mean Cell Hemoglobin, Mean Cell Hemoglobin Concentration, Platelets, Red Cell Distribution Width, and Mean Platelet Volume. Differentials were also done.

### **Statistical Analysis**

The data were analyzed using a one-way ANOVA (differential and hematology data), two-factor ANOVA without replication (blood chemistry data), and the two-factor ANOVA with replication (kaolin consumption data: Data Analysis ToolPak Software, Microsoft Office-Excel 2010. *P* values < 0.05 were considered significant, and *P* values < 0.001 were considered highly significant.

## **Results**

The thirteen samples of kaolin soil that were purchased from stores in Alabama and Georgia are shown in Table 1. The most commonly sold brand was “Best White Dirt” from Phenix City, AL. The brand that was sold in the largest supply was “Granny Own White Dirt” of unknown origin.

### **Physical and Chemical Determination**

#### ***Munsell Chart***

The Munsell color notations of the kaolin samples ranged from white to pink. Six samples exhibited white color with Munsell notations of either 10YR 8/1 or 5YR 8/1. Four samples were characterized as very pale brown colors with Munsell notations in the 10YR 8/2. Two samples had pink colors with Munsell notations of 5YR 8/3 and 5YR 7/4, and one sample had a pale yellow color with Munsell notation of 2.5YR 8/2 (Table 1). The pH of the samples ranged from 3.85 (acidic) to 7.63 (basic). The pale yellow sample with a Munsell color notation of 2.5YR 8/2 had the strongest acid reaction of 3.85 (Table 1). The other 12 samples had pH over 5 and three white samples had pH values that exceeded 7. Samples that had color in the very pale brown to pale yellow color spectrums had very strong acidic pH values.

#### ***Microscopic Analysis***

The TEM micrograph showed that there were two distinct types of particles present in the kaolin (data not shown). Most of the particles were irregular in shape and were~ 300-500nm in size.

Table 1. List of Kaolin Sample, Results of Munsell Comparisons, and Soil pH (n=13) [(1)]

<b>ID</b>	<b>Kaolin Supplier</b>	<b>Hue</b>	<b>Location</b>	<b>Value</b>	<b>Chroma</b>	<b>Soil Color Name</b>	<b>pH</b>
1	Traditional Products	10YR	Phenix, City AL	8	2	Very Pale Brown	4.46
2	White Dirt	5YR	Georgia	8	1	White	6.9
3	Home Grown Georgia	2.5Y	Georgia	8	2	Pale Yellow	3.85
4	Down Home Georgia White Dirt	10YR	Georgia	8	1	White	7.44
5	Best White Dirt	10YR	Unknown	8	2	Very Pale Brown	4.68
6	Best White Dirt	5YR	Phenix City AL	8	3	Pink	4.63
7	Best White Clay	10YR	Phenix City, AL	8	3	Very Pale Brown	4.42
8	Down Home Georgia	5YR	Griffin, Georgia	8	1	White	7.13
9	Best White Clay	10YR	Phenix City, AL	8	2	Very Pale Brown	4.64
10	Home Grown Georgia White Dirt	5YR	Georgia	8	1	White	4.74
11	Unknown White Dirt	10YR	Unknown	8	1	White	5.25
12	Granny's Own	5YR	Unknown	8	1	White	7.63
13	Unmarked Kaolin Soil	5YR	Unknown	7	4	Pink	4.47

The other particles were spherical in shape and measured ~50-100nm. The results of the EDS analysis (data not shown) showed that the kaolin contained Al, Si, Fe, C, and Cu. Since the Cu and C were expected from the TEM grid, these elements were not considered in estimating the atomic percentages of the elements present in the sample (44.2%, Al; 23.5%, Si; and 6.7%, Fe). A second sample was tested on a molybdenum grid to rule out the possibility that the C and Ca came from the grid. The results showed that the kaolin contained Zn (0.9%), Cu (29.6%), Al (2.9%), Si (5.6%), Fe (3.9), Ca (10.8%), C (12.5%), Cr (5.7%), K (0.7%), Cl (6.9%), P (3.2%), Mg (2.0%), S (5.6%), and O (9.7%). Since the Mo was expected from this TEM grid, Mo was not considered in estimating the percentages of the elements present in the sample.

***Elemental Analysis***

All kaolin samples contained the nutrients (in order of abundance) iron, calcium, magnesium, potassium, sodium, boron, phosphorus, and sulfur (Table 2). The toxins arsenic, cadmium, and lead were present in high amounts in the samples tested. The kaolin samples originating in Georgia had the highest levels of the elements tested (Table 2). Sample #3, from Home Grown Georgia White Dirt had the highest levels of all elements. Sample #1, from Traditional Products, Phenix City, AL, had the highest amount of iron of any kaolin sample.

Table 2. Soil Elemental Analysis (ppm, n=13)

<b>ID</b>	<b>Ca</b>	<b>K</b>	<b>P</b>	<b>As</b>	<b>Ba</b>	<b>Cd</b>	<b>Cr</b>	<b>Cu</b>	<b>Fe</b>	<b>Mn</b>	<b>Mo*</b>	<b>Ni</b>	<b>Pb</b>
<b>1</b>	122.6	27.9	70.9	5.6	5.9	0.4	32.7	13.8	7185.5	0.5	ND	0.3	9.8
<b>2</b>	1556.8	71.7	199.2	0.4	92.0	0.1	14.7	11.9	1141.4	5.4	ND	2.6	23.1
<b>3</b>	3302.6	2312.5	346.1	14.9	47.6	12.2	19.0	19.3	1593.9	24.9	33.9	22.0	31.1
<b>4</b>	1506.8	224.2	138.7	1.3	37.7	0.6	9.6	10.9	890.1	13.7	0.1	2.3	13.8
<b>5</b>	2204	759.5	229.1	3.4	28.2	1.0	23.8	15.3	2694.0	40.3	ND	4.5	7.8
<b>6</b>	938	227.3	93.9	2.3	11.9	0.4	25.2	10.3	1172.2	15.1	ND	3.3	1.8
<b>7</b>	273.5	135.8	49.4	0.5	9.5	0.1	20.4	10.1	1187.6	5.0	ND	1.9	4.8
<b>8</b>	1774.2	79.2	66.1	1.2	21.2	0.1	10.8	10.2	484.4	4.3	0.7	2.9	16.3
<b>9</b>	188.5	38.8	118.5	0.8	8.3	0.1	26.1	23.4	1278.6	2.7	ND	4.3	2.0
<b>10</b>	2138.2	721.4	264.4	4.6	122.8	2.4	11.6	14.6	929.6	8.2	5.9	4.9	17.1
<b>12</b>	1501.2	160.9	61.1	1.3	23.2	0.1	10.6	11.7	864.5	4.8	ND	1.3	8.2
<b>13</b>	470.8	127.5	75.3	0.8	253	0.1	25.0	10.4	751.5	1.6	ND	2	7.7
<b>Ave</b>	<b>1229.0</b>	<b>375.9</b>	<b>131.7</b>	<b>2.84</b>	<b>50.9</b>	<b>1.36</b>	<b>17.7</b>	<b>12.5</b>	<b>1518.0</b>	<b>9.73</b>	<b>3.07</b>	<b>4.03</b>	<b>11.03</b>

\* ND=Not Detected

Ca= Calcium, K=Potassium, P=Phosphorus, As=Arsenic, , Ba=Barium, Cd=Cadmium, Cr= Chromium, Cu= Copper, Fe= Iron, Mn= Manganese, Mo= Molybdenum, Ni= Nickel, , Pb= Lead,

\*\*Note: Other elements were tested but not displayed due to publication space: Selenium, Boron, Sodium, Zinc



## Kaolin Consumption Study

### Mouse Behavior

Mice in the Kaolin 1:1 Chow group were phenotypically undistinguishable from mice in the control (no kaolin) group. Mice in the All Kaolin group appeared smaller and sicker, compared to the other treatment groups, and had to be separated by Day 4 to prevent cannibalism. Eventually, the experiment was halted to prevent mortality in this group. Mice in the No Kaolin group were alert, playful, and aware, compared to the other treatment groups.

### Kaolin Consumption

Kaolin consumption between treatment groups was not significantly different ( $P = 0.181$ ). The amount of kaolin consumed by mice in the stationary groups (Table 3) ranged from 2.8g to 14.6g per mouse, with the average being 9.9g for Kaolin 1:1 and 6.1g for All Kaolin. The amount of kaolin consumed by stationary mice was more than the kaolin consumed by mice in the rotation group (Kaolin 1:1R). Mice that were individually housed, consumed more kaolin (average = 13.1) than mice that were housed in a group (average = 3.53), regardless of gender.

Table 3. Kaolin Experiment Set Up and Consumption Week One Post-treatment (n=48)

Cage	Mouse/Cage Gender	Ratio Kaolin	Kaolin Consumed	Chow Difference	Kaolin Consumed/ Mouse	Chow Consumed / Mouse
1	1 F	Kaolin 1:1	14.2	33.9	14.20	33.90
2	4 F	Kaolin 1:1	16.9	48.7	4.23	12.18
3	1 M	Kaolin 1:1	11.7	23.0	11.70	23.00
4	1 F	Kaolin 1:1	14.6	23.2	14.60	23.20
5	4 M	Kaolin 1:1	11.3	72.1	2.83	18.03
6	1 M	Kaolin 1:1	11.9	25.9	11.90	25.90
7	2 F	All Kaolin	18.0	--	9.00	--
8	4 M	All Kaolin	17.3	--	4.33	--
9	4 F	All Kaolin	11.2	--	2.80	--
10	2 M	All Kaolin	16.9	--	8.45	--
11	2 M	No Kaolin	--	--	--	--
12	4 M	No Kaolin	--	--	--	--
13	4 F	No Kaolin	--	--	--	--
14	2 F	No Kaolin	--	--	--	--
15	4 F	All Kaolin (rotational)	13.8	--	3.45	--
16	2 F	All Kaolin (rotational)	9.17	--	4.59	--
17	3 F	Kaolin 1:1 (rotational)	9.02	56.6	3.01	18.87
18	3 F	Kaolin 1:1 (rotational)	11.1	62.405	3.70	20.80

\*Kaolin consumption between treatment groups was not significantly different ( $p = 0.181$ )

### Electrolytes

Electrolyte panels were not significantly different between treatment groups ( $P = 0.395$ ).



**Kidney Panel.** Creatine kinase was highest in the Kaolin 1:1R group (4,074 U/L) and lowest in the All Kaolin group (981 U/L). Amylase was not present in the No Kaolin and Kaolin 1:1 R groups, but was higher in the Kaolin 1:1 group (818 U/L) than in the All Kaolin group (3U/L). The total protein in the No Kaolin group was the highest, and the lowest level was in the All Kaolin group [not shown in Table]. The Total Bilirubin levels in the mice were highest in the No Kaolin group (9.2mg/dl) and lowest in the Kaolin 1:1 group (2.8mg/dl). [not shown in Table].

### **Hematology**

The treatments were not significantly different from each other ( $P=0.367$ ) in terms of hematology values. The white blood cell count (WBC) (Table 5) was lowest in the No Kaolin group (0.24 K/ul). The Kaolin 1:1 R group had the largest WBC count at 3.82 K/ul. Hemoglobin was lowest in the “No Kaolin” group (11.1 K/ul) and the highest in the All Kaolin group (17.4) K/ul (Table 5). The Hematocrit count was lowest in the Kaolin 1:1 R group and highest in the All Kaolin group at 89.3% (not shown in Table). The mean cell volume count was lowest in the All Kaolin group and the highest in the Kaolin 1:1 R group at 99.1 f/L (not shown in Table). Mean Cell Hemoglobin was lowest in the All Kaolin group at 16.8pg and highest in the Kaolin 1:1 R group at 64.7pg (not shown in Table). Platelets were the lowest in the No Kaolin group at 138 K/ul and highest in the All Kaolin group at 791 K/ul (Table 5). Neutrophils were highest for the All Kaolin group, 0.52 k/ul and lowest for the No Kaolin group at 0.01 K/ul (Table 5).

Table 5. Hematology. Complete Blood Count Analysis (n=12/Group)

<b>Treatment</b>	<b>WBC</b>	<b>NEU</b>	<b>LYM</b>	<b>MONO</b>	<b>EOS</b>	<b>BASO</b>	<b>RBC</b>	<b>HGB</b>	<b>RDW</b>	<b>PLT</b>
<b>Units&gt;</b>	K/ $\mu$ l	K/ $\mu$ l	K/ $\mu$ l	K/ $\mu$ l	K/ $\mu$ l	K/ $\mu$ l	M/ $\mu$ l	g/dl	%	K/ $\mu$ l
All Kaolin	1.06	0.52	0.95	0.03	0.19	0.00	10.3	17.4	20.5	791
Kaolin 1:1	0.43	0.02	0.36	0.01	0.03	0.01	17.3	12.1	19.7	143
Kaolin 1:1 R	3.82	0.38	2.46	0.76	2.46	0.38	2.28	14.8	18.0	409
No Kaolin	0.24	0.01	0.16	0.02	0.02	0.03	6.18	11.1	18.9	138

WBC=White Blood Cell	NEU=Neutrophils	LYM=Lymphocytes,	MONO=Monocytes
EOS=Eosinophils	BASO=Basophils	RBC=Red Blood Cell	HGB=Hemoglobin
PLT=Platelet	RDW=Red Cell Distribution Width		
K/uL=1000Xn per microliter	M/uL= 10 <sup>6</sup> X n per microliter	fL=fentoliter	pg=picogram

\*Hematology values were not significantly different ( $p = 0.367$ ) between treatment groups

Differentials revealed much lower percentages of neutrophils in all groups, compared to normal values obtained for the murine wild-type strain. However, treatment groups were not significantly different from each other ( $P=0.999$ ). Lymphocyte percentages were similar to normal values but monocytes, eosinophils and basophils were higher than normal values.

## **Discussion**

### **Physical and Chemical Determination: Munsell Chart**

Most of the samples exhibiting white colors originated from Georgia while the very pale brown samples all originated from Alabama. This suggests that the geology of the area affected the chemical constituents of the kaolin samples. The colors also suggest that impurities in kaolin might be mainly iron oxides, resulting in the pale brownish colors observed. The pH values suggest that the mineralogical composition of the samples differed greatly. Since acidic pH values in soil are mainly attributed to the hydrolysis of the aluminum-hexahydrate ions, the samples exhibiting the low pH values may have high Al content. Low pH also tends to make micronutrients and heavy metals more soluble. The fact that most of the samples had a pH above 5.0 suggests that the mineral elements besides Al and Si in these samples tended to fall on the basic side. The relationship of the colors to the pH suggests that the samples from different areas may have different toxicological or pharmacological reactions.

### **Microscopic Analysis**

Using the Cu grid, the TEM and EDS analyses revealed high amounts of Al and Si in the kaolin, which also contained a high level of Fe. The Mo grid tests showed that kaolin had a high percentage of copper and other metals. However, it is important to note that the analyses only looked at nanoscale portions of the kaolin sample. The kaolin was not homogenous, but rather contained various elements, which were present in different amounts in each 100nm-sized area.

### **Elemental Analysis**

Elemental analysis suggested that a kaolin-only diet lacked adequate levels of essential nutrients but contained some trace elements in excessive amounts. For example, kaolin samples from “Home Grown Georgia White Dirt,” had the highest levels of micronutrients compared to the other soil samples. The trace elements of greatest concern were arsenic, cadmium, and lead, which occurred at concentrations of 14.9, 12.2, and 31.1 ppm, respectively. The mean background levels of these elements in U.S. soils are 7.4 ppm for As, 1.1 ppm for Cd and 20 ppm for Pb (IARC, 2001; Kabata-Pendias and Pendias, 1984; USGS, 1974). In the “Home Grown Georgia White Dirt” sample, the levels of As and Cd were 2 to 10 times that of the average US background levels. Lead was 36% higher than the background concentrations found in US soils. Furthermore, neither As, Cd, nor Pb has been found to be essential in humans (USEPA, 2001); these elements are actually detrimental for life processes. For example, As and Cd are among the top 5 most toxic trace elements on the United States Environmental Protection Agency’s (EPA) list of toxic substances, and are classified as known human and animal carcinogens (Barton et al., 1992; IARC, 1993). Chronic oral exposure of humans to inorganic arsenic at doses as low as .05-0.1 mg/kg/day is frequently associated with neurological or hematological toxicity (Goddard et al., 1992; Prasad and Rossi, 1995; Sass et al., 1993; NSF International, 2003).

Iron, arsenic, cadmium, and lead were found in all kaolin samples tested, although the bioavailability of these in the kaolin samples was not tested. The U.S. Food and Drug

Administration (DHHS, 2007) has suggested a mean lifetime exposure to Cd from all food sources to be 0.14 ug/kg/day for a 70 kg adult. Elevated concentrations of Cd in foodstuffs have been associated with kidney disorders (DHHS, 2007). Chronic exposure to Pb has been shown to cause damage to the brain, kidneys, and circulatory systems. Those at greatest risks are children and pregnant women, the same groups often associated with kaolin geophagia.

## **Mice**

The mouse model used in this study may have affected results. We used an IL-10 deficient mouse model (129SvEv IL-10<sup>-/-</sup>) because these mice are more sensitive to stressful conditions, like the rotating cage, and more likely to experience gastrointestinal distress, due to the lack of immunological tolerance and perturbations in gastrointestinal flora. However, the blood chemistry and hematological data might have been altered by their genetic mutation.

## **Consumption Data**

The Kaolin 1:1 (rotational) group consumed the least amount of kaolin, which was surprising. Preliminary data had indicated that rotating the mice significantly increased kaolin consumption (Kitts, 2010). However, the preliminary data were obtained from a short, 3-day study, and any initial differences that might have been found in the preliminary study may have been masked over the longer treatment time used in this study. Historically, rotation is known to increase kaolin consumption, and has been used as an indirect measure of rodent toxicity and nausea (Santucci et al., 2002; Yamamoto et al., 2002; Liu et al., 2006). It is possible that the rotational device or protocol was not effective in producing gastrointestinal distress in the mice. There is other data to suggest that mice do not always evidence rotational-related pica (References for Lab Animals, 2009). Consumption was greatly increased by isolation. The presence of even one additional mouse in a cage removed this isolation effect. Kaolin consumption was accompanied by increased chow consumption.

## **Blood Chemistry and Hematology**

### ***Electrolytes***

Sodium levels were consistent with normal mouse values (Tseng, 2004). Chloride levels were similar for the “No Kaolin” and “Kaolin 1:1” groups. Carbon dioxide levels were much greater in the “Kaolin 1:1” groups compared to both the “All Kaolin” and “No Kaolin” groups. Higher carbon dioxide levels indicate increased alkalinity in the mice in these groups.

### ***Kidney Panel***

Glucose levels were much greater in mice in the kaolin stationary groups compared to rotated mice and “No Kaolin” controls. This may indicate the presence of a carbon source in the kaolin samples, which might explain the euphoria and satisfaction felt after consumption by people practicing kaolin geophagia (unpublished data). Components of kaolin, like Zn, Pb and As, might block insulin release or insulin receptors, resulting in higher glucose blood levels. The arsenate form of arsenic can substitute phosphate in the formation of phosphate intermediates involved in glucose metabolism, which could slow down glucose metabolism, interrupt the production of energy, and interfere with ATP-dependent insulin secretion. The arsenite form of arsenic has high affinity for sulfhydryl groups, and thus, can form covalent bonds with the disulfide bridges in insulin, insulin receptors, glucose transporters, and enzymes involved in glucose metabolism. As a result, the normal functions of these molecules can be hampered

(Mouse Phenome Database, 2013). The evidence suggests a health risk for consuming kaolin, especially by pregnant mothers. The high consumption puts them at risk for developing diabetes mellitus during and after pregnancy. Calcium levels were similar across all groups in the kaolin consumption tests. This suggests that the calcium was not bioavailable. Phosphorus levels were much lower in groups that consumed kaolin compared to the no kaolin control group. Creatinine levels were higher in the stationary kaolin groups, but lower in the kaolin rotational group compared to no kaolin controls. Albumin levels were lower among the groups consuming kaolin compared to controls. This suggests that kaolin consumption may modulate kidney function.

### ***Liver Panel***

Alanine Aminotransferase levels, which indicate potential disorders with liver function, were similar across all groups tested. AST levels, which indicate muscle, liver or heart damage, were lower in all of the stationary kaolin groups, compared to control mice. The total bilirubin levels of mice in the kaolin groups were similar to the levels seen in control mice. The total protein levels, used to determine liver or kidney function, were much lower in the kaolin groups compared to the “No Kaolin” control groups. This is consistent with the fact that mice replaced a portion of their diet with a non-protein food source. Cholesterol levels were lower in the stationary kaolin groups compared to “No Kaolin” controls. Creatine kinase levels were higher in all of the kaolin groups, compared to the control, indicating a potential for cardiomyopathies.

### ***Hematology***

White blood cells, neutrophils, and lymphocytes were increased in mice in the kaolin consumption groups. This may indicate infection or other immunological disturbances in mice on kaolin diets. Platelets were increased as well, which might indicate blood loss or liver disease. Other complete blood cell count parameters were unchanged or fluctuated between groups.

### **Conclusion**

Kaolin samples contained a wide variety of elements with biological relevance. While some nutritive elements may not be present in adequate quantities, other toxic elements may be present in excessive amounts. However, the bioavailability of these elements is not known and needs to be assessed. Kaolin consumption affected mouse behavior and liver/renal panels with the greatest impact being on triglycerides, alanine aminotransferase, glucose and bilirubin levels.

Hematology results showed increased levels of white blood cells, lymphocytes, neutrophils and platelets, which suggests that kaolin consumption is associated with immunological problems, to potentially include infections. This could also have been related to the inability of the genetically altered IL-10<sup>-/-</sup> mouse model to develop immunological tolerance and to mount an appropriate immunological defense. Additional studies, with larger animal numbers and a different mouse strain, might add to the understanding of the relevance of kaolin geophagia on health.

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