Peanut CRSP Final Report for Project UGA145

FINAL SUMMARY

A. Goals: Aflatoxins and fumonisins are ubiquitous foodborne toxicants and the co-occurrence of these mycotoxins in human foods represents a significant public health concern, which has been strongly associated with human aflatoxicosis, neural tube defects, as well as many types of primary cancers. The long-term goal of this project is to establish a global network for assessment of aflatoxin (AF) and fumonisin (FN) exposure in high-risk populations and to develop effective intervention strategies for diminishing incidence and mortality of human mycotoxicoses. The establishment of such a network will allow to further evaluate the linkage between AF and FN exposure and human nutrition deficiency and to identify roles of AF and FN exposure in aggravation of infectious diseases. The immediate goal of this research proposal is to develop highly sensitive, non-antibody, non-radioactive, and non-mass spectrometry (MS) based analytical methods for rapidly measuring serum AFB_1-lysine (AFB-LYS) adduct and FN biomarkers. In addition, this project provided analysis for measuring serum AFB_1-albumin adduct in samples collected by Projects TAM 149, UAB148, and COR158.

B. SIGNIFICANT TECHNICAL ACHIEVEMENTS:

1. Establishment of Methods to Measure Serum AFB_1-Lysine (AFB-LYS) Adduct

An HPLC method has been established and procedures include Pronase digestion, solid-phase (Oasis) concentration and purification, and fluorescent detection; limit of detection is 0.1 pg/mL serum or 10 fg/mg albumin; recovery is ranged 75-90% for various spiked concentrations; and reproducibility and accuracy are excellent.

2. Integrative Toxicopathological Evaluation of AFB_1 Exposure in Animals

An integrative evaluation of the toxicopathological effects of AFB_1 in F344 rats was conducted. Briefly, male F344 rats were orally exposed to a single-dose of AFB_1 at 0, 50, 250 or 1000 μg/kg body weight (BW) or repeated-dose of AFB_1 at 0, 5, 10, 25 or 75 μg/kg BW for up to 5 weeks. The growth of animals, as represented by body weight gain, was inhibited in high dose AFB_1-treated animals. Biochemical and histological changes were assessed together with the formation of AFB_1-lysine adduct (AFB-Lys) in serum and liver foci positive for placental form glutathione S transferase (GST-P^+). In single-dose protocol, serum AST, ALT and ALP activities were dose-dependently elevated with maximal changes (> 100 folds) appeared at 3-day after treatment. A transient increase of AFB-Lys adduct appeared at 2 h after treatment. Animal that received 250 μg/kg AFB_1 showed concurrent bile duct proliferation, necrosis and appearance of GST-P^+ hepatocytes at 3-day while the preneoplastic GST-P^+ foci appeared after 1-week. Neither liver GST-P^+ hepatocytes nor foci were induced by 50 μg/kg AFB_1 treatment. In repeated-dose protocol, bile duct proliferation and liver GST-P^+ foci co-occurred after 3-week, followed by proliferation foci formation after 4-week and dramatic ALT, AST and CK elevations after 5-week exposure in animals received 75 μg/kg AFB_1. Liver GST-P^+ hepatocytes and foci appeared in a dose- and time-dependent manner, low dose of AFB_1 (5 μg/kg) did not induce liver GST-P^+ foci formation throughout the experiment. Serum AFB-Lys increased temporally at low doses (5-

25 μg/kg) and reached a maximum after 2-week exposure at 75 μg/kg group followed by a gradual decrease, consistent with liver histological changes that may affect the adduct formation. This integrative study demonstrates that liver GST-P^+ cells and foci are sensitive biomarkers for AFB_1 toxic effect and correlated with bile duct proliferation and biochemical alterations in F344 rats, which hold promise as potential target for future intervention strategies.

3. Investigation of Immune Toxic Effects of AF exposure in Animals

F-344 rats (140-160 g) from Harlan were treated with single dose (0, 50, 250, 750 μg/kg of AFB_1) and repeated doses (0, 5, 25, and 75 μg/kg of AFB_1). Animals were killed at the different time points: for single-dose study 2, 24, 48, 72 hours, and 7 days; for repeated-dose study 3 days, 1, 2, 3, 4, and 5 weeks. At each time point, five rats of each group were included. Blood and urine samples were collected for evaluation of aflatoxin biomarkers. Major organs: liver, lung, kidney, heart, stomach, and intestine were collected, half of these organs were frozen in liquid nitrogen for molecular biological studies and the other half organs were fixed in 10% buffered formalin solution for histopathological evaluation. Spleen and thymus were collected for both H.E. staining and cryopreservation. Specifically, we completed the evaluation on AFB_1-induced changes in splenic lymphocyte phenotypes and inflammatory cytokines expression in male F344 rats. In addition, serum AFB_1-lysine adducts level and liver and spleen histology were examined to correlate with possible immune effects. One-week treatment with AFB_1 dose-dependently decreased the percentages of splenic $CD8^+$ T cells and $CD3^-CD8a^+$ NK cells and the expressions of IL-4 and IFN-γ by $CD4^+$ T cells, IL-4 and IFN-γ by $CD8a^+$ cells, and TNF-α by NK cells; however, no concurrent histological changes in liver or spleen tissues were found, suggesting acute inhibition without structural alteration. Five-week treatment with AFB_1 significantly increased the percentages of $CD3^+$ and $CD8^+$ T cells. The anti-inflammatory cytokine IL-4 expression by $CD4^+$ T cells was significantly decreased and significant increases of pro-inflammatory cytokine IFN-γ expression by $CD4^+$ T cells and TNF-α expression by NK cells were also found, indicating inflammatory responses and apoptosis involved. Consistent with this finding, liver periportal necrosis and bile duct proliferation as well as splenic lymphocyte apoptosis were found especially at high dose. Serum AFB_1-lysine increased by about two-fold at low doses but not at the highest dose, consistent with liver injure observed. Our data demonstrate that AFB_1 exposure can modulate expression of both splenic lymphocyte phenotypes and cytokines.

4. Measurement of Serum AFB-LYS Adduct for Global Human Exposure

In this funding period, we evaluated AF exposure in serum samples collected from Many countries, including US samples from the San Antonio (SA) Environmental Health Study (n=151, collected by TAM 149), a case-control study in Guangxi (n=204), China; Ghanaian mothers (n=755, collected by UAB148) and infants (n=71, collected by UAB 148), and Haitians (n=193, collected by COR158). The detection rate of AFB-lysine adduct is 17.2% in SA population, 91.2% in Guangxi liver cancer cases, 97.1% in Guangxi controls, 90.7% in Ghanaian mothers, 79% in Ghanaian infants, and 76.2% in Haitians, respectively. Levels of AFB-lysine adduct in SA population averaged 0.89 ± 2.08 (SD) with the median 0.15, ranged 0.05-16.01 pg/mg albumin; in Guangxi liver cancer cases averaged 10.60 ± 28.21 with the median 4.97, ranged 0.54-227.13 pg/mg albumin; in Guangxi controls averaged 7.34 ± 16.56 with the median 3.69, ranged 0.28-175.83 pg/mg albumin; in Ghanaian mothers averaged 10.86 ± 19.01 with the

median 5.01, ranged 0.44-286.73 pg/mg albumin; in Ghanaian infants averaged 2.36 ± 2.44 with the median 1.46, ranged 0.14-10.02 pg/mg albumin; and in Haitian averaged 4.17 ± 12.09 with the median 1.17, ranged 0.16-130.39 pg/mg albumin. From these data, Ghanaian mothers and Guangxi liver cancer cases had high level of exposure to AFs; Guangxi liver cancer cases had higher AF exposures than the matched controls ($p<0.001$); Ghanaian infants and Haitian had median level of AF exposure; whereas the SA population at the US had the minimal exposure to AF.

We also completed analysis of AFB-LYS adduct in a total of 1305 serum samples previously collected in a cross-sectional study (n=812) and an intervention study (n=493) by Dr. Philippe Nikiema from high-risk populations in Burkina Faso. Although the preliminary results from portion of these samples were reported last year, the whole datasets have been collected and evaluated by biostatistical methods in this year. The cross-sectional study included residents of 3 villages (one from central area and two from west areas) and 2 waves of sample collections. Levels of AFB-Lys of the first wave collection (Sep-Oct 2000) showed significantly statistical differences on the median levels of AFB-Lys were found among villages, with the village Sourkoudingan (n=160; median=17.5; range: 2.8-627.8 pg/mg albumin) having higher adduct level ($p<0.001$) as compared to villages Campela (n=184; median=10.0; range: 0.5-978.7 pg/mg albumin) and Boende (n=138; median=10.2; range: 0.8-135.6 pg/mg albumin). The different exposure patterns were found in the second wave of sample collections (Apr-May 2001) in the same 3 villages, with the central village Campela (n=106; median 12.1; range: 0.5-192.4 pg/mg albumin) having higher adduct levels ($p<0.001$) as compared to west villages, Sourkoudingan (n=115; median=6.4; range: 0.4-493.9 pg/mg albumin) and Boende (n=109; median=6.9; range: 1.5-174.0 pg/mg albumin). The intervention study was conducted in two different west villages with Badala as the control and Diofoloma as intervened. Intervention protocol used was adapted from Dr. Wild including education and grossly hand pick-up for moldy corn and ground nuts. Intervention period was 10-month and aflatoxin exposure was evaluated through measurement of AFB-Lys adduct in blood samples collected from residents of the intervened and control villages. The median levels of AFB-Lys adduct are similar in both villages before the intervention initiated (13.51 vs. 13.87 pg/mg albumin). After 10-month intervention, the median AFB-Lys level decreased about 14.6% in intervened group (14.18 pg/mg albumin) as compared to the control group (16.60 pg/mg albumin) with no statistical significance ($p=0.774$). These data showed that low efficacy for the intervention strategy adapted.

Analysis of AFB-LYS adduct was also conducted in serum samples recently collected from a cross-sectional study (n=175) in Malaysia. The detection rate of AFB-LYS in these samples is 98.0% and levels of AFB-LYS averaged 6.31 ± 3.54 with the median 6.08, ranged 0.81-13.67 pg/mg albumin, suggesting a higher exposure in residents of the country. Further analysis of AFB-LYS adduct was also conducted in serum samples collected by Dr. Pauline Jolly's group (UAB148) from their HIV study subjects (n=315) in Ghana. The detection rate of AFB-LYS in these samples is 96.0% and levels of AFB-LYS averaged 15.78 ± 15.64 with the median 11.26, ranged 0.48-109.87 pg/mg albumin, suggesting a very higher exposure in these patients of the country.

In the last funding year we collaborated with British Medical Research Council Uganda Unit and RAKAI Health Program and measured AFB1-lysine adduct in human serum samples (n=1931) from two existing cohort studies conducted in Uganda. In samples (n=725) from the General

Population Cohort (GPC), approximate 90% (651/725) had detectable AFB1-lysine adduct with a median of 1.61 pg/mg albumin and ranged from 0.40 to 253.11 pg/mg albumin. In samples (n=1,206) from Rakai Community Cohort Study (RCCS), 85% (1025/1206) had detectable AFB1-lysine adduct with a median of 3.19 pg/mg albumin and ranged from 0.41 to 167.04 pg/mg albumin. Levels of AFB1-lysine adduct in the RCCS samples are significantly higher than those in the GPC samples ($p<0.05$). Temporal pattern of AFB1-lysine adduct in these two distinct human populations were further evaluated in 3-year interval since the initiation of cohorts. Detection rate of AFB1-lysine adduct in GPC is varied: 94% in 1989, 100% in 1992, 84% in 1995, 80% in 1998, 72% in 2001, 93% in 2004, 97% in 2007, and 94% in 2010 with median (range) of 1.77 (0.43-22.46), 2.44 (1.02-5.06), 0.83 (0.41-18.60), 0.89 (0.41-6.02), 1.15 (0.41-11.55), 1.77 (0.41-14.80), 1.92 (0.40-30.89), and 2.29 (0.47-253.11) pg/mg albumin, respectively. Detection rate of AFB1-lysine adduct in RCCS is varied: 71% in 1994, 96% in 1996, 91% in 1999, 50% in 2002, 92% in 2005, 89% in 2008, and 90% in 2011 with median (range) of 2.44 (0.41-94.79), 1.16 (0.41-555.33), 1.19 (0.45-122.51), 5.08 (0.51-30.92), 3.58 (0.41-67.56), 3.22 (0.45-117.96), and 2.60 (0.43-167.04) pg/mg albumin, respectively. We also measured samples AFB1-lysine adduct levels in samples collected from other studies conducted in Uganda. Our results clearly demonstrated temporal pattern of AF exposure in rural human populations of Uganda.

5. Development of Analytical Method for Studying Fumonisin Exposure in Animals and Human Populations

In this funding period, a specific and sensitive method based on HPLC-fluorescence has been developed for the determination of FB1 biomarkers. Major efforts were put on sample preparation to maximize recovery and minimize interference of endogenetic components in biospecimen. The procedure essentially involved an immunoaffinity column followed by solid-phase extraction. Extracts were derivatized using o-phthaldialdehyde (OPA), and OPA derivatives were separated with a reversed-phased C18 column and detected at an excitation wavelength of 340 nm and an emission wavelength of 455 nm. FB1 biomarkers in extracts without OPA derivatization was unambiguously confirmed with liquid chromatography-tandem mass spectrometry using electrospray ionization and multiple reaction monitoring. The HPLC method was validated according to the European Commission Decision 2002/657/EC. The limits of detection and limits of quantitation were found to be 0.1 ng/mL and 0.5 ng/mL, respectively. The accuracy and precision were determined, with recoveries in the range of 85% to 95% and the relative standard deviation less than 7% at spiked levels of 1.0 to 10.0 ng/mL.

To validate the method developed for measurement of FB_1 biomarkers, F344 rats were administered by *gavage* with either a single dose of 0, 10 or 25 mg FB_1/kg body weight (BW) or repeated doses of 0, 1.0, or 2.5 mg FB_1/kg BW/day for 5 weeks. FB_1 excretion and FB_1-induced metabolic alterations of sphingolipids in rat urine, feces, and serum were assessed. Dose-dependent urinary and fecal excretion of free FB_1 was found in both single-dose- and repeat-dose-treated rats. In the single-dose study, urinary sphinganine (Sa) to sphingosine (So) ratio (Sa/So) reached a maximum at day 7 for the high-dose group and at day 5 for the low-dose group, whereas serum Sa/So showed only marginal changes. In the repeat-dose study, urinary Sa/So was persistently elevated at 2 weeks, while serum Sa/So was unchanged. Time-course changes of sphinganine 1-phosphate (SaP) and sphingosine 1-phosphate (SoP) were also

examined. Although serum Sa/So and SaP/SoP ratios showed no signs of time- or dose-dependent changes, a 10-fold increase in urinary SaP/SoP was observed, suggesting that urinary SaP/SoP is a more sensitive biomarker for FB_1 exposure. The accumulation of SaP and SoP was evident in the time-course of SaP/Sa and SoP/So, which may reflect activity changes of enzymes closely related to the metabolism and catabolism of SaP and SoP. These results provide concrete evidence towards the practical use of excreted FB_1, Sa/So, and SaP/SoP as biomarkers of exposure to FNs.

We further measured sphingolipids in human samples using the methodology we previously developed. Human serum and urine samples from Huaian (n=43), a high-risk area of esophageal cancer, and Fusui (n=33), a high-risk area of liver cancer, were analyzed for Sa and So by HPLC-fluorescence. The median serum Sa level in Fusui subjects was significantly higher than in Huaian subjects (13.04 vs. 7.82 nmol l^{-1}, $p<0.01$). Also, the serum Sa/So ratio was significantly higher in Fusui subjects than in Huaian subjects (0.78 vs. 0.41, $p<0.01$). The median So level in Fusui subjects was 17.95 nmol l^{-1}, which was comparable to the level in Huaian subjects (17.67 nmol l^{-1}) ($p=0.76$). For urinary sphingolipid metabolites levels in study subjects, a significantly higher median level of urinary Sa was detected in Fusui subjects than in Huaian subjects (3.79 vs. 2.45 nmol l^{-1}, $p=0.01$). The median Sa/So ratio in Fusui subjects was also higher than in Huaian subjects (0.57 vs. 0.31, $p<0.01$). However, Huaian subjects had a higher level of urinary So than Fusui subjects (7.69 vs. 4.74 nmol l^{-1}, $p<0.01$). We could not detect SaP and SoP in our human samples.

To evaluate association between FB1 exposure and human disease risks and to assess application of FB1 biomarkers in human populations, we conducted a population-based case-control study consisting of 190 ESCC cases and 380 age-, gender-, residency- matched controls in Huaian, one of the highest incidence for esophageal squamous cell carcinoma (ESCC) in China.. Urinary free FB1 and sphinganine/sphingosine (Sa/So) ratio were measured by HPLC-fluorescence detection. Odds ratio (ORs) and 95% confidence intervals (CI) were calculated for association between urinary FB1 biomarkers and ESCC risk using conditional regression models. Urinary free FB1 was significantly higher in cases (median: 176.13 pg/mg creatinine) than in controls (median: 56.92) ($p<0.01$). The adjusted ORs (95% CI) of ESCC risk for moderate vs. low and high vs. low urinary free FB1 level were 2.71 (1.43 – 5.14) and 5.90 (3.16 – 11.01), respectively (P-trend <0.01). In subjects consumed corn meal, urinary free FB1 was significantly correlated with estimated corn meal intake in both controls (rSpearman = 0.20, N = 245, $p<0.01$) and cases (rSpearman = 0.22, N = 140, $p<0.01$). Urinary Sa/So ratio didn't significantly differ between cases and controls (median: 0.23 vs. 0.19, $p=0.35$). Adjusted ORs (95% CI) for moderate vs. low and high vs. low urinary Sa/So ratio level were 1.69 (0.94 – 3.04) and 2.32 (1.27 – 4.24), respectively (P-trend <0.01). These results indicate association of urinary free FB1 level and increased ESCC risk and suggest urinary free FB1 as FB1 exposure biomarker in human study.

6. Investigation of Co-Exposure of AFB1 and FB1 in Human Populations

In this funding period, the co-contamination of AFB_1 and FB_1 in food and human dietary exposure was investigated in residents of three different areas of China. A total of 209 food samples were measured for AFB_1 and FB_1. The median AFB_1 levels were 13.5, 2.3 and 1.3 $mgkg^{-1}$ and the median FB_1 levels were 2.6, 0.4 and 0.3 $mgkg^{-1}$ in corn samples collected from

Huaian (a high-risk area for esophageal cancer), Fusui (a high-risk area for liver cancer) and Huantai (a low-risk area for both esophageal and liver cancers), respectively. The median level of AFB_1 in plant oil of Fusui was the highest (52.3 $mgkg^{-1}$) among all food samples analyzed. Co-contamination of these two mycotoxins was found in corn, rice and wheat flour. Based on measured food consumption data, the averaged daily dietary intake of AFB_1 was 0.397 mg (range: 0.269–1.218 mg) in residents of Huantai, 1.723 mg (0.224–49.772 mg) in Huaian, and 2.685 mg (1.006–14.534 mg) in Fusui. Residents in Huaian and Fusui had the higher dietary exposure to AFB_1 than that in Huantai ($p<0.001$). The averaged FB_1 daily dietary intake was 92.4 mg (range: 55.0–362.1 mg) for residents of Huantai, 460.0 mg (83.2–2894.5 mg) in Huaian, and 138.6 mg (30.0–10,541.6 mg) in Fusui. Residents in Huaian had significantly higher FB1 exposure than those in Huantai and Fusui ($p<0.001$), and residents in Fusui had a significantly higher FB_1 exposure than those in Huantai ($p<0.05$). The percentages of residents whose daily FB_1 intakes were higher than 120 mg, the provisional maximum tolerable daily intakes (PMTDI) established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (2001), were significantly different among three areas, with 36.1%, 95.3% and 64.7% in residents of Huantai, Huaian, and Fusui, respectively. These data suggest that the co-exposure to AFB_1 and FB_1 in residents of rural China may contribute to the etiology of human chronic diseases in high-risk areas.

C. SIGNIFICANT ISSUES/CHALLENGES

The most significant issues in this project over past five year funding period are that we developed highly sensitive methods for measurement of AFB1 and FB1 biomarkers and validated these methods in animal studies. We further used these methods to screen serum samples from seven countries and tried to establish a global network for human AF exposure. Human populations in West Africa exposed to the highest amount of AF via their corn and groundnuts based diet. We also conducted animal studies to explore the growth inhibition and immune toxic effects of AF exposure. In addition, methods for measuring FB1 biomarker were also developed.

The great challenge is how to prevent human exposure, especially in the host Africa countries and the other challenge is lack of advanced techniques and instruments. We originally planned to hold two training courses in Ghana and Uganda; however, due to the lack of equipment and supplies, we had to bring scientist and technical people from host countries to US laboratory for training.

D. CAPACITY DEVELOPMENT

In this funding period, we successfully established biomarker-based methods for studying AF and FN exposure in US laboratories. We applied these methods to screening over 5,000 samples from 7 countries with 5 host countries with USAID support. We helped the establishment of a laboratory in Ghana and trained people from Ghana, Burkina Faso, Uganda, and Malaysia.

E. HUMAN CAPACITY/TRAINING

Name	Gender	Country	Degree	Completion Date	Where Trained	Employment of Trainee
Afriyie-Gyawu, E	M	Ghana	PhD at TAMU	Oct. 2007; Mar. 2008, training in Wang's lab	TTU	Georgia Southern U.
Wang, P.	M	China	PhD	Jan. 2008	TTU	UCLA
Wang, Z.	M	China	PhD	May 2008	TTU	U of Indiana
Johnson, N. M	F	US	PhD at TAMU	Mar.2009 Training in Wang's lab	UGA	Johns Hopkins U.
Li Xu	F	China	PhD	Aug 2010	UGA	U. of Texas
Muwanika, R	M	Uganda	6-months Training	June 2012	UGA	Rakai Health Program, Uganda
Massey, M	M	US	BS (Two years training in Wang's lab)	May 2012	UGA	US CDC
Wang, Franklin	M	US	MS	May 2012	UGA	County Health Dept.
Guoqing Qian	M	China	PhD	Dec 2012	UGA	Emory U.

F. KEYWORKSHOPS/SHORT-TERM TRAININGS

Name	Gender	Location of Training	Host Country	Length of Training	Training Type
Ankrah, N-A.	M	TTU, USA	Ghana	3 weeks	Aflatoxin measurement

Nikiema, P. A	M	UGA	Burkina Faso	9 months	AFB-Albumin adduct measurement
Nkurunziza, P	M	UGA	Uganda	6 months	AFB-Albumin adduct measurement
Muwanika, R	M	UGA	Uganda	6 Months	AFB-Albumin adduct measurement
Nkwata, A. K	M	UGA	Uganda	9 months	Detection of Mycotoxins

G. Publications and Presentations:

1. Jiang, Y., **Jolly, P. E**., Preko, P. O., **Wang, J.-S**., **Ellis, W. O., Williams, J. H. and Phillips, T. D**. (**2008**) Aflatoxin related immune dysfunction in health and in human immunodeficiency virus disease. *Clinic. Develop. Immunol.* Article ID 790309, doi:10.1155/2008/790309.
2. Afriyie-Gyawu, E**.,** Wang, Z., **Ankrah, N-A**., Johnson, N. M., Tang, L., Xu, L., Ofosuhene, M., Kumi, J., Huebner, H. J., **Jolly, P. E., Ellis**, Taylor, R., Brattin, B., W. O., **Ofori-Adjei, D., Williams, J. H., Wang, J-S., and Phillips, T. D**. (**2008**). Clinical intervention trial with NovaSil clay in Ghanaians at high risk for aflatoxicosis: Nutrient utilization and non-nutritional factors. *Food Additives & Contam*, 25 (7):872-884.
3. Wang, P., Afriyie-Gyawu, E., Tang, Y., Johnson, N. M., Xu, L., Tang, L., Huebner, H. J., **Ankrah, N.-A., Ofori-Adjei, D., Ellis, W., Jolly, P. E., Williams, J. H., Wang, J.-S., Phillips, T. D.** (**2008**). NovaSil clay intervention in Ghanaians at high risk for aflatoxicosis: II. Reduction in biomarkers of aflatoxin exposure in blood and urine, *Food Additives & Contam*, 25 (5):622-634.
4. **Phillips, T. D**., Afriyie-Gyawu, E., **Williams, J**., Huebner, H., **Ankrah, N-A., Ofori-Adjei, D., Jolly, P.**, Johnson, N., Taylor, J., Marroquin-Cardona, A., Xu, L., Tang, L., **Wang, J.-S.** (**2008**). Reducing Human Exposure to Aflatoxin through Use of Clay. *Food Additives & Contam*, 25(2):134-145.
5. Afriyie-Gyawu, E., **Ankrah, N.-A**., Huebner, H. J., Ofosuhene, M., Kumi, J., Johnson, N. M., Tang, L., Xu, L., **Jolly, P.E., Ellis, W. O., Ofori-Adjei, D., Williams, J. H., Wang, J.-S. & Phillips, T. D.** (**2008**). NovaSil clay intervention in Ghanaians at high risk for aflatoxicosis. I. Study design and clinical outcomes. *Food Additives & Contam*, 25(1):76-87.
6. Xu, L., Tang, L., Qian, G., Su, J. and **Wang, J.-S**. (2009). Predominance of aflatoxin exposure in rural residents of Southern Guangxi, China. *Toxicologist*, 104, 754.
7. Qian, G., Tang, L., Xu, L., Johnson, N. M., Tietze, D., Rodriguez, M., Kaufman, L., Cunningham, K., Wittmer, J., Guerra, F., Donelly, K. C., **Phillips, T. D., and Wang, J.-S.**

(2009). Serum level of aflatoxin B1-lysine adduct in a US population compared to a high risk population in China. *Toxicologist,* 104, 755.

8. Xu, L., Afriyie-Gyawu, E., Jiang, Y., Tang, L., Huebner, H. J., **Ankrah, N.-A., Ofori-Adjei, D., Ellis, W. O., Jolly, P. E., Williams, J. H., Wang, J.-S., Phillips, T. D**. **(2008).** NovaSil clay intervention in Ghanaians at high risk for aflatoxicosis: Immune parameters as biomarker of effect. *Toxicologist*, 102, 490.
9. Tang, L., Xu, L., Afriyie-Gyawu, E., Liu, W., Wang, P., Tang, Y., Wang, Z., Huebner, H. J., **Ankrah, N.-A., Ofori-Adjei, D., Williams, J. H., Wang, J.-S and Phillips, T. D**. (2009). Aflatoxin-albumin adducts and correlation with decreased serum levels of vitamins A and E in an adult Ghanaian population. *Food Additives and Contam.* 26 (1): 108–118.
10. Xu, L., Cai, Q., Tang, L., Hu, X., Sun, G., and **Wang, J.-S.** (**2009**) Dietary fumonisin B_1 exposure and human esophageal cancer risk in Huaian, China: Use of urinary free fumonisin B_1 as biomarker of exposure. *Proc. AACR*, **50**, 3013.
11. **Wang, J.-S**. (2009). Aflatoxin exposure and nutrition in developing countries. Presentation to USAID, January 14, 2009 in Washington, DC.
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13. Xu, L., Qian, G., Tang, L., Su, J., and **Wang, J.-S**. (2010). Genetic Variations of Hepatitis B Virus and Serum Aflatoxin-Lysine Adduct on High Risk of Hepatocellular Carcinoma in Southern Guangxi, China. *J. Hepatol*. 53:671-676.
14. Xu, L., Cai, Q., Tang, L., Wang, S., Hu, X., Su, J., Sun, G., **Wang, J.-S. (2010).** Evaluation of fumonisin biomarkers in a cross-sectional study with two high-Risk populations in China. *Food Additive & Contam.* 27, 1161-1169.
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17. Tang, L., Qian, G., Xu, L., Johnson, N., **Jolly, P., Williams, J. H., Phillips, T. D**. and **Wang, J.-S. (2010).** Initiation of global network for aflatoxin exposure studies. Toxicologist, 114, 1457.
18. Qian, G., Tang, L., Liu, W., and **Wang, J.-S**. **(2010).** Development of a non-antibody method for rapid detection of serum aflatoxin B1-lysine adduct. *Toxicologist*, 114, 1163.
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FINAL INTERPRETATION

A. IMPORTANCE OF TECHNICAL ACHIEVEMENTS

In this funding period, we developed methods for measurement of aflatoxin and fumonisin biomarkers. We conducted animal studies for validation of these methods and investigated the growth inhibition and immune toxic effects in animals. We measured over 5,000 blood samples from various host countries, including Ghana, Burkina Faso, Uganda, Haiti, and Malaysia. Based on our other studies in other countries, including China and USA, we able to establish a biomarker based global network for aflatoxin exposure.

B. IMPORTANCE OF PHYSICAL AND HUMAN CAPACITY DEVELOPMENT

In this funding period, this project trained 6 PhD students, 1 MS student, and 1 BS student. They are all working in different Institutions of US.

C. HERITAGE LEFT FROM WORKSHOPS AND SHORT-TERM TRAINING

In this funding period, we trained 5 scientists from Ghana, Burkina Faso, and Uganda with the training period of 3 weeks to 9 months. We planned to have two training courses in Ghana and Uganda, but due to lack of equipment/instrument or supplies, the plan had been changed to bring these people to UGA for a long-term training.

D. HERITAGE LEFT IN PUBLICATIONS

There are 5-6 publications left from the project, including collaborative projects in human population studies.

FINAL SUMMARY OF ACCOMPLISHMENTS BY OBJECTIVES

This project has completed all objectives proposed in 2007-2012 phase and has provided analysis for measuring serum AFB_1-albumin adduct in samples collected by Projects TAM 149, UAB148, and COR158, as originally planned.