

1. Final Summary

a. Brief statement of overall goal

The overall goal of the project outlined for **objectives 1-6** is to determine the effect of aflatoxin exposure in food on immune and health status of HIV negative and HIV positive people and the association of aflatoxin with progression to HIV disease in HIV positive people. This work is highly novel and is the first that we know of that will provide empirical data on aflatoxin exposure and HIV disease. The longitudinal design of this study provides certain strengths over our previous studies since it allows us to: avoid bias in measuring exposure; determine time sequence between exposure and disease progression; study multiple exposures and multiple outcomes; and calculate progression of disease in relation to level of exposure. For **Objective 7**, we conducted a cross-sectional study among 785 pregnant women to investigate the association of aflatoxin levels with maternal anemia and adverse birth outcomes.

b. Significant Technical Achievements-Host Country, US?

Objective #	Technical Achievement – Host Country	Technical Achievement – US
<p>1. Association between aflatoxin and socio-demographic/economic characteristics of HIV positive people</p>	<p>The study showed that several socio-demographic/economic factors such as, educational level, ethnic group, the village in which participants lived, number of individuals in the household, and number of children in the household attending secondary school, were significantly associated with high AFB1 albumin-adduct (AF-ALB) levels. <i>These findings indicate strongly that post-harvest and food handling and preparation interventions can be implemented among the different socio-economic and ethnic groups in Ghana to reduce aflatoxin exposure.</i></p>	<p>US stakeholders in aflatoxin contamination of crops can benefit from this information. Also, USAID and US researchers can use the information as they plan to provide further assistance to subsistence farming communities in Ghana and elsewhere in the world that struggle with the aflatoxin contamination of staple food crops.</p>
<p>2. Association between aflatoxin and health and clinical status</p>	<p>The study in HIV negative people, showed statistically significant associations between high aflatoxin levels and symptoms of acute aflatoxicosis, abnormal liver function and hepatitis B virus (HBV) infection. <i>This finding of the association of high levels of aflatoxin with symptoms of acute aflatoxicosis in a population that also has high levels of hepatitis infection and abnormal liver function indicate that a high</i></p>	<p>The health or clinical factors identified in this study is beneficial to US clinicians and veterinarians as they provide care to their clients. The information can also serve as a basis for further research and assistance by the US investigators and the USAID.</p>

	<p><i>proportion of the population is at significant risk of developing liver cancer.</i></p> <p>Among HIV positive people, the study showed that those with high aflatoxin levels had statistically significant increased risk of having higher HIV viral loads, higher bilirubin and lower albumin levels. <i>Thus, aflatoxin exposure may contribute to high viral loads and abnormal liver function in HIV-positive people and so promote disease progression. Presentation of these health/clinical factors as predictors of high AF-ALB levels provides them with routine methods for suspecting/diagnosing aflatoxin poisoning in patients in their daily clinical work.</i></p>	
<p>3. Association between aflatoxin and micronutrient status in HIV- and HIV+ Ghanaians.</p>	<p>The study demonstrated that participants with high AF-ALB had significantly lower vitamin A concentrations and marginally lower vitamin E. Conversely, AFM₁ was positively associated with vitamin A and vitamin E. <i>These data indicate that aflatoxin may modify plasma micronutrient status and that preventing aflatoxin exposure may greatly reduce micronutrient deficiencies in developing countries such as Ghana.</i></p>	<p>Identification of the association between aflatoxin levels and micronutrient deficiency is beneficial to US clinicians and veterinarians as they provide care to their clients. The information can also serve as a basis for further research and assistance by the US to developing countries with high aflatoxin contamination of crops.</p>
<p>4. Identify immune impairments associated with aflatoxin levels in HIV+ individuals.</p>	<p>The study showed that high aflatoxin levels were associated with HIV-associated changes in T cell phenotypes and B cells among HIV positive people. For example, the loss of T regulatory cells (Tregs) in HIV positive people with high aflatoxin levels may facilitate HIV associated immune hyperactivation and lead to more severe disease and disease progression. <i>By identifying the effect of aflatoxin on HIV immune and clinical status,</i></p>	<p>These are the first data on the association between aflatoxin and HIV disease and they have significance for treatment of HIV positive people in the US who may encounter immune suppression from fungal toxins. This also has implications for US funding for HIV treatment programs in developing countries since the</p>

	<p><i>appropriate and targeted strategies can be implemented widely in Ghana and other developing country populations worldwide to decrease aflatoxin intake and hence decrease the rate of progression of HIV disease in infected people. Further, because of the immune suppressive and other harmful health effects of aflatoxin, the effect of antiretroviral therapy (ART) in delaying AIDS may not be as great in populations that are exposed to the toxin as it is in industrialized countries. The results also raise the question of the benefit of current vaccines (and of a potential HIV vaccine) to people in aflatoxin exposed regions of the world since aflatoxin suppresses the immune responses to vaccinations. For all of these reasons, this study of HIV disease progression associated with aflatoxin exposure is a highly significant and area of research.</i></p>	<p>drugs may not be as effective in countries where there are factors such a mycotoxins that counteract the beneficial effects of the treatments. These data should be considered seriously as health donors and officials implement and carry out plans to address the HIV/AIDS epidemic.</p>
<p>5. Examine the association between aflatoxin levels and progression of HIV infection to AIDS</p>	<p>We found that HIV positive people had significantly higher levels of AF-ALB than HIV negative people. This finding suggests that HIV positive people may accumulate higher levels of AF-ALB in their blood. <i>Thus, aflatoxin exposure may contribute to high viral loads in HIV-positive persons and promote HIV disease progression. This finding should be taken into account in clinical care of HIV positive people in the country. This may contribute to earlier achievement of Millennium Development Goal #6 (Combat HIV/AIDS, malaria and other diseases).</i></p>	<p>These are the first data on the association between aflatoxin and HIV disease and they have significance for treatment of HIV positive people in the US who may encounter immune suppression from fungal toxins.</p>
<p>6. Association between aflatoxin and active tuberculosis infection in HIV+ people.</p>	<p>The study showed that participants in the highest AF-ALB quartile had significantly higher Hazard Ratios for developing symptomatic TB compared to those in the lowest quartile. <i>Therefore, high aflatoxin levels seem to predispose HIV</i></p>	<p>This is the first study to show association between aflatoxin and development of symptomatic TB in HIV positive people and the finding also has significance for treatment of HIV positive</p>

	<p><i>positive people to development of symptomatic TB. This finding is highly significant for clinical care of HIV+ people in Ghana and other parts of sub-Saharan Africa that face aflatoxin contamination of staple food crops and high rates of TB. Addressing this problem may contribute to earlier achievement of Millennium Development Goal #6 (Combat HIV/AIDS, malaria and other diseases).</i></p>	<p>people in the US who may encounter immune suppression from fungal toxins.</p>
<p>7. Association between aflatoxin levels, health status, anemia and adverse birth outcomes in pregnant women</p>	<p>This study found strong associations between aflatoxin and maternal anemia and between aflatoxin and adverse birth outcomes. <i>We are the first group to report the finding of an association between aflatoxin and anemia.</i> Low birth weight predisposes infants to adverse growth and developmental outcomes resulting in severe morbidity and mortality. Thus, our findings have practical implications for policy makers in developing countries to put in place documented methods to reduce aflatoxin exposure in their populations. The findings also have implications for targeted nutritional education of pregnant women in areas with high levels of aflatoxin contamination of foods. This may contribute to achievement of Millennium Development Goals #s 4 & 5 which relate to reduction of infant and maternal mortality, respectively.</p>	<p>We have provided new data on the association between aflatoxin and anemia. Although iron deficiency anemia (IDA) is the most common cause of anemia there are many other causes including aflatoxin exposure. Consequently, any assessment of anemia must take all of the possible causes into consideration if appropriate multi-disciplinary interventions are to be instituted to address the problem. Our finding on the association between aflatoxin and low birth weight infants adds to the growing body of evidence that shows association between low birth weight and aflatoxin exposure.</p> <p>These findings are important to the USAID and to US researchers who can use the information as they plan to provide further assistance to maternal and child health programs in developing countries such as Ghana that struggle with the aflatoxin contamination problem.</p>

		Currently, there is great interest among US and international researchers and groups such as the GATES FDN on the role of mycotoxins in stunting in infants especially in developing countries.
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a. Significant Issues/Challenges

The biggest challenge that this program has experienced is lack of sufficient and continuous funding for biomarker and other laboratory tests (which are quite expensive) to be completed. It has also been difficult to get funding from the National Institutes of Health since the National Institute of Allergy and Infectious Diseases (NIAID) sees aflatoxin and HIV as an environmental matter and the National Institute of Environmental Health Sciences (NIEHS) sees it as an infectious disease problem. Consequently, several grant applications submitted to the NIH with data gathered with Peanut CRSP funding have not been funded. The PI has trained US students funded by her NIH international research training grant and other UAB students to collect data for the study over the years.

b. Capacity development, i.e. laboratory, field, equipment-Host Country, US

We have provided the field site in Ghana with VICAM equipment for testing aflatoxin in grains and with a -20 freezer for storage of samples. We have worked with the laboratories of the Kumasi Center for Collaborative Research in Tropical Diseases on the campus of the Kwame Nkrumah University of Science and Technology (KNUST) to process blood and urine samples and have trained Ghanaian Research Assistants to collect data collection from study participants at hospital and community sites. The training of US students at all levels (undergraduates, master’s and doctoral) has allowed performance of all the studies with collection of a significant amount of meaningful data. This has produced a cadre of researchers in this field that understands health problems in developing countries and will contribute toward reducing and ultimately eliminating health disparities among groups in the US and abroad.

c. Human Capacity/Training Table

Name	Sex	Country	Degree	Completion date	Country trained	Employment
Francis Obuseh	Male	Nigeria/US	MPH & DrPH	2008	Ghana	US Army Captain U.S. Air Force Surgeon General from Ramstein Air Base
Wendy Keys	Female	US	MPH	2003	Ghana	Nurse Practitioner
Tara Loughlin	Female	US	MPH	2003	Ghana	Nurse Practitioner
Inas Mahdi	Female	US	MPH	2005	Ghana	Evaluation/Dissemination

						Specialist, Population Service International
Maribel Diaz	Female	US	BS	2008	Ghana	Master's program in health
Dorothy Hammond	Female	Ghana	DrPH	Transferred to UGA	Ghana	
Nelly Yatich	Female	Kenya	DrPH	2009	Ghana	Assist. Prof. Univ. Of Washington stationed in Nairobi
Aimen Ismail	Female	US	MD		Ghana	Medical student at UAB
Naomi Chen	Female	US	MPH	2010	Ghana	PhD program – University of Texas Health Sciences Center, School of Public Health
Farah Srichandra	Female	US	BS	2012	Ghana	Master of Public Health student
Angele Marandet	Female	US	MPH	2012	Ghana	Global Surveillance Fellow, Division of Global HIV/AIDS (DGHA), CDC
Michelle Joseph	Female	US	BS	2011	Ghana	Public Health Associate with the CDC in Miami, Florida
Rasheedah Godfrey	Female	US	BS	2011	Ghana	Tutor and Volunteer at St. Bernardine's Community Hospital
Faisal Shuaib	Male	Nigeria	DrPH	2010	Ghana	Works with GATES FDN. in Nigeria
Dnika Joseph	Female	US	MPH	2011	Ghana	Manager -Girl Scouts of North Central Alabama
Andrea Gonzalez	Female	US	BS	2012	Ghana	Medical School – University of Puerto Rico
Roshni Sheth	Female	US		BS Expected May 2013	Ghana	Senior- University of Alabama at Birmingham
Abena Afriyie	Female	US		MPH Expected May 2013	Ghana	MPH program - University of Alabama at Birmingham
Justin Hills	Male	US		BS expected May 2013	Ghana	Undergrad student – North Carolina State Univ.
Raymond Hunt	Male	US		MD degree expected 2014	Ghana	UAB Medical student
John Keenan	Male	US	PhD	Still in program	US	PhD student - UAB

d. Key workshops/short-term trainings listed in a table that includes:

Location of Training	Training Type	Number of M/F
Ghana	Research Assistants – Interview participants, collect data, keep records	4 Females and 2 Male (Olivia, Christy, Christy, Lizzy, Joshua and Eric)
Ghana	Participant recruitment and study methodology	7 Females 1 Male (3 nurses, 1 Physician Assist., 2 doctors and 2 data entry clerks)

Over the years we employed and trained 6 full time Ghanaian research assistants (4 females and 2 males) in research methods. These research assistants have been trained in pilot testing questionnaires, data collection and recording, quality control, and medical data abstraction. Three nurses, a Physician Assistant, 2 doctors and two data entry personnel at the clinics have also participated in training on participant recruitment and study methodology.

e. Publications since 2007

1. **Jolly, P.E.**, Jiang, Y., Ellis, W.O., Appawu, J., Awuah, R.T., Nnedu, O., Adjei, O., Stiles, J., Person, S., and Jolly, C.M. Association between aflatoxin levels, health characteristics, liver function, hepatitis and malaria infections in Ghanaians. Journal of Nutritional and Environmental Medicine, 16:1-16, 2007.
2. Dash, B., Afriyie-Gyawu, E. Hubner, H.J., Porter, W., Wang, J.S., **Jolly, P.E.** and Phillips, T.D. Determinants of the variability of aflatoxin-albumin adduct levels in Ghanaians Journal of Toxicology and Environmental Health, Part A, 70: 58-66, 2007.
3. Jiang Y., **Jolly, P.E.**, Preko, P., Baidoo, J., Wang, J-S., Ellis, W.O., and Williams, J.H. Aflatoxin related immune dysfunction in Health and in Human immunodeficiency virus diseases. Clinical and Developmental Immunology, 2008, Article ID 790309, doi:10.1155/2008/790309.
4. Shuaib, F.M., Ehiri, J., and Williams, J.H., Abdullahi, A. and **Jolly, P.E.** Reproductive health effects of aflatoxins: A review of the literature. Reproductive Toxicology, 29 (3) 2010; doi:10.1016/j.reprotox.2009.12.005.
5. Shuaib, F.M., **Jolly, P.E.**, Yatich, N., Jiang, Y., Ehiri, J., Funkhouser, E., Person, S., Wilson, C., Ellis, W.O., Wang, J.S., and Williams, J.H. Association between birth outcomes and aflatoxin B₁ biomarker blood levels in pregnant women in Kumasi, Ghana, Tropical Medicine and International Health, 15:160-167, 2010.

6. Shuaib, F.M.B., **Jolly, P.E.**, Ehiri, J.E., Jiang, Y., Ellis, W.O., Stiles, J.K., Yatich, N.J., Funkhouser, E., Person, S.D., Wilson, C., and Williams, J.H. Association between anemia and aflatoxin B₁ biomarker levels among pregnant women in Kumasi, Ghana. American Journal of Tropical Medicine and Hygiene. 83:1077-1083, 2010. doi:10.4269/ajtmh.2010.09-0772.
7. Keenan, J., **Jolly, P.E.**, Preko, P., Baidoo, J., Jiang, Y., McGwin, G. Jr. Association between aflatoxin B1 albumin adduct levels and tuberculosis infection among HIV+ Ghanaians. Archives of Clinical Microbiology, 2011, Vol. 2, No. 3:3, doi:10:3823/230.
8. Obuseh, F., **Jolly, P.E.**, Jiang, Y., Shuaib, F., Waterbor, J., Ellis, W.O., Piyathilake, C., Ellis W.O., Desmond, R.A., Afriyie-Gyawu, E., and Phillips, T. Relationship between aflatoxin B1 albumin adducts in plasma, aflatoxin M1 in urine and vitamin A and E levels in Ghanaians. International Journal of Vitamin and Nutrition Research 80:355-368, 2010.
9. **Jolly, P.E.**, Shuaib, F., Jiang, Y., Preko, P.O., Baidoo, J., Stiles, J.K., Wang, J-S., Phillips, T.D, and Williams, J.H. Association of viral load and liver function with high aflatoxin levels in HIV positive Ghanaians. Food Additives and Contaminants Part A. 2011 Sep; 28(9):1224-34. Epub 2011 July 12.
10. Obuseh, F., **Jolly, P.E.**, Preko, P.O., Kulczycki A., Ehiri J., Waterbor J., Jiang, Y., Piyathilake C.J. Desmond R.A. Aflatoxin exposure and health characteristics associated with vitamin A and E levels in HIV positive Ghanaians. Journal of the International AIDS Society, 2011, 14:53 doi:10.1186/1758-2652-14-53.
11. Shuaib, FMB, Ehiri, JE, Yatich, NJ, Funkhouser, E., Person, SD, Wilson, C., Turpin, C., Agbenyega, T., Williams, JH, Qian, G., Wang, J-S., and **Jolly, PE**. Socio-demographic determinants of aflatoxin B₁-lysine adduct levels among pregnant women in Kumasi, Ghana (in press, Ghana Medical Journal).

Publications with other PNUT CRSP Investigators

1. Afriyie-Gyawu, E., Wang, Z., Ankrah, N.A., Xu, L., Johnson, N.M., Tang, L., Guan, H., Heubner, H.J., **Jolly, P.E.**, Ellis, W.O., Taylor, R., Brattin, B., Ofori-Adjei, D., Williams, J.H., Wang, J-S., and Phillips, T.D. NovaSil clay does not affect bioavailability and utilization of vitamins A and E and minerals in Ghanaians at high risk for aflatoxicosis. Food Additives and Contaminants, 25(7): 872-884, 2008.
2. Wang, P., Afriyie-Gyawu, E., Tang, Y., Johnson, N., Xu, L., Tang, L., Heubner, H.J., Ankrah, N-A., Ofori-Adjei, D., Ellis, W.O., Jolly, **P.E.**, Williams, J.H., Wang, J.S., and Phillips, T.D. NovaSil clay intervention in Ghanaians at high risk for aflatoxicosis: II Reduction in biomarkers of aflatoxin exposure in blood and urine. Food Additives and Contaminants, 25 (5): 622-634, 2008.
3. Phillips, T.D., Afriyie-Gyawu, E., Williams, J.H., Heubner, H.J., Ankrah, N.-A., Ofori-Adjei, D., **Jolly, P.E.**, Johnson, N., Taylor, J., Marroquin-Cardona, A., Xu, L., Tang, L., Wang, J.-S. Reducing human exposure to aflatoxin through the use of clay: A review. Food Additives and Contaminants, 25: 134-145, 2008.
4. Afriyie-Gyawu, E., Ankrah, N.A., Heubner, 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., and Phillips, T.D. NovaSil clay intervention in Ghanaians at high risk for aflatoxicosis: Part I. Study design and clinical outcomes. Food Additives and Contaminants, 25: 76-87, 2008.

5. Johnson, N.M., Afriyie-Gyawu, E., Huebner, H., Marroquin-Cardonaa, A., Robinson, A., Tangb,L., Xu, L., Ankrah, N-A, Ofori-Adjei, D., **Jolly,P.E.**, Williams,J.H., Wang,J-S., Phillips, T.D. PAH exposure in a Ghanaian population at high risk of aflatoxicosis. Science of the Total Environment, 407: 1886-1891, 2009.
6. Bley N'Dede, C. Jolly, C. M., Vodouhe, D. S., and **Jolly, P.E.**. Economic Risks of Aflatoxin contamination in Marketing of Peanut in Benin. Economics Research International, 2012, Article ID 230638, 12 pages. doi:10.1155/2012/230638.
7. Robinson, A., Johnson, N.M., Strey, A., Taylor, J.F., Marroquin-Cardona, A., Mitchell, N. J., Afriyie-Gyawu, E., Ankrah, N-A., Williams, J.H., Wang, J-S., **Jolly, P.E.**, Nachman, R., and Phillips, T.D. Calcium montmorillonite clay reduces urinary biomarkers of fumonisin B₁ exposure in rats and humans Food Additives and Contaminants, Part A, Chem Anal Control Expo Risk Assess 29: 809-818, 2012.

Offshoot publications:

1. Yatich, N., Funkhouser, E., Ehiri, J., Agbenyega, T., Stiles, J.K., Rayner, J., Turpin, A., Ellis, W.O., Jiang, Y., Williams, J.H., Afriyie-Gwayu, E. and Phillips, T.D. and **Jolly, P.E.** Malaria and intestinal helminths and other risk factors for stillbirth in Ghana, Infectious Diseases in Obstetrics and Gynecology, Article ID 350763, doi:10.1155/2010/350763, 2010.
2. Yatich, N. J., **Jolly, P.E.**, Funkhouser, E., Agbenyega, T., Rayner, J. C., Ehiri, J. E., Turpin, A., Stiles, J. K., Ellis, W.O., Jiang, Y., and Williams, J.H. The effect of malaria and intestinal helminth coinfection on birth outcomes in Kumasi, Ghana, American Journal of Tropical Medicine and Hygiene, 82:28-34, 2010.
3. Yatich, N., Jiang, Y., Agbenyega, T., Turpin, A., Ellis, W.O., Funkhouser, E., Ehiri, J., Rayner, J., Williams, J.H., and **Jolly, P.E.** Malaria and intestinal helminth co-infection among pregnant women in Ghana: Prevalence and risk factors. American Journal Tropical Medicine and Hygiene, 80: 896-901, 2009.
4. Wilson, N.O., Bythwood, T., Solomon, W., **Jolly, P.**, Yatich, N.J., Jiang, Y., Shuaib, F., Adjei, A., Anderson, W., Stiles, J. K. Elevated levels of IL-10 and G-CSF associated with asymptomatic malaria in pregnant women, Infectious Diseases in Obstetrics and Gynecology, 2010, Article ID 317430, 7 pages, doi:10.1155/2010/317430.

Invited Presentations in 2012

1. Invited to speak on “Association of aflatoxin and viral load in HIV infected people” at the joint meeting of the 7th Conference of The World Mycotoxin Forum[®] and the XIIIth IUPAC International Symposium on Mycotoxins and Phycotoxins in Rotterdam, the Netherlands, 5-9 November 2012. This unique combined event, ‘WMFmeetsIUPAC’, will build on the success of the previous conferences which were held separately all over the world.
2. Invited to the Bill and Melinda GATES meeting on “AFLATOXIN: IMPACT ON STUNTING IN CHILDREN AND INTERVENTIONS TO REDUCE EXPOSURE” to make presentation on the “Role and mechanisms of aflatoxin in maternal anemia, low birth weight and stunting among infants and children in Africa”. Meeting Location and date: International Food Policy Research Institute, 2033 K St., NW, Washington, DC 20006, February 1-2, 2012.

3. Invited to make presentation at the United States Agency for International Development, Washington, DC on the “Importance of Mycotoxins to public health in developing countries”, March 13, 2012. This meeting was attended by USAID technical staff and external invitees.

2. Final Interpretation

a. Importance of Technical Achievements-

- i. Significant aflatoxin and health information was provided to the Host Country that can be used to reduce aflatoxin exposure and improve the health of the population. These could lead to earlier achievement of Millennium Development Goals # 6 (*Combat HIV/AIDS, malaria and other diseases*), and #s 4 and 5 (*Reduce child mortality and Improve maternal health*).
- ii. The aflatoxin and health information can be used by US stakeholders in aflatoxin contamination of crops and by the USAID and US researchers as they plan to provide further assistance to subsistence farming communities in developing countries that struggle with the aflatoxin contamination of staple food crops. The information on the health or clinical predictors of high aflatoxin levels is beneficial to US clinicians and veterinarians as they provide care to their clients.

b. Importance of physical and human capacity development-

- i. Several Host Country people have been trained as in research methods and as research assistants. They will be available to conduct or participate in the appropriate conduct of future research. Foreign doctoral trainees from Kenya and Nigeria have returned to assume significant positions in their countries and will be able to assist in tackling the aflatoxin/mycotoxin food and health problem. One of these graduates now works with the GATES Foundation in Nigeria. Another of our doctoral graduates is US Army Capt. **Francis Obuseh** who is with U.S. Air Force Surgeon General from Ramstein Air Base and an officer in charge of the humanitarian and civic assistance operations for Southern Accord and Medlite 2012. Captain Obuseh recently participated in promoting HIV screening and safe male circumcision in Botswana as part of the MEDLITE/SOUTHERN ACCORD 12 exercise, a key element in a broader series of military-to-military activities that demonstrate the strong partnership between the U.S. and Botswana defense forces. "We're here as part of MEDLITE/SOUTHERN ACCORD 12," said **Capt. Francis Obuseh**, officer-in-charge of the HCA event. "As part of the exercise, we are providing humanitarian assistance to support the BDF in achieving their goals in different medical aspects."
- ii. Approximately 17 US students have been trained in various aspects of aflatoxin research and in overall field, clinical and laboratory research methods in Ghana. This has provided a cadre of US researchers that understands health problems in developing countries and will contribute toward reducing and ultimately eliminating health disparities among groups in the US and abroad.

c. Heritage left from workshops and short-term training-

- i. As we conducted our studies we provided our Host Country participants with information on aflatoxin, the factors that promote build-up of this toxin in food and prevention methods. These studies were conducted in full partnership with our host country collaborators. Therefore, the results of the studies are available for use in development of educational, agricultural and health interventions to improve the health of the people in Ghana.
- ii. US students have been trained in research methods and in aflatoxin research and have participated in training in-country research assistants, farmers and study participants.

d. Heritage left in publications

Our long list of publications shows that a significant heritage has been left in publications that will benefit our host country and documents US contribution.

3. Final Summary of Accomplishments by Objective - 2007-2012

Objective 1: Determine the association between aflatoxin biomarker levels and socio-demographic/economic characteristics of HIV positive people in Ghana.

We have entered the baseline sociodemographic/economic and aflatoxin B1 data for the 300 HIV positive participants we recruited into an access database. We received the aflatoxin B1 levels for plasma samples collected at 5-8 month and 12 months post-recruitment from Dr. Jia Sheng-Wang in January 2013. We have been unable to complete the analyses for this aim due to disruption in funding. Once funding is restored we will develop a measure that can be used to accurately divide the population into high and low AFB1 groups for data analyses and analyze the data for the association between AFB1 levels and socio-demographic/economic characteristics of the study group. In 2006 we published a manuscript on association between aflatoxin biomarker levels and socio-demographic/economic characteristics of HIV negative people in Ghana (**Jolly, P.E., Jiang, Y., Ellis, W.O., Awuah, R.T., Nnedu, O., Wang, J., Phillips, T., Afyie-Gyawu, E., Person, S., and Jolly, C.M.** *Determinants of aflatoxin levels in Ghanaians: Sociodemographic factors, knowledge of aflatoxin and food handling and consumption practices. International Journal of Hygiene and Environmental Health, 209: 345-358, 2006*).

Objective 2: Determine the association between aflatoxin biomarker levels and health and clinical status (hepatitis B and C infections, malaria Ag, liver function, CD4 count, HIV viral load, opportunistic infections) HIV positive people in Ghana.

The baseline and all follow-up clinical data for the 3-year time period have been entered into an access database. These include data from tests conducted for hepatitis B infection, malaria antigen and liver function, clinical data collected for participants at baseline to obtain HIV diagnosis date, viral load and CD4+ T cell count as well as any clinical diagnoses and medications prescribed. Patients' medical records were reviewed at 5-8 months, 12-16 months, 24 months and 36 months and clinical information collected. These data will be analyzed to determine the association between AFB1 levels and clinical status of the participants. We did not have enough funds to conduct tests

for Hepatitis C Virus infection. In 2007 we published a paper on aflatoxin biomarker levels and health and clinical status in HIV negative people (*Jolly, P.E., Jiang, Y., Ellis, W.O., Appawu, J., Awuah, R.T., Nnedu, O., Adjei, O., Stiles, J., Person, S., and Jolly, C.M. Association between aflatoxin levels, health characteristics, liver function, hepatitis and malaria infections in Ghanaians. Journal of Nutritional and Environmental Medicine, 16:1-16, 2007*).

Objective 3: Determine the association between aflatoxin biomarker levels and micronutrient status (vitamin A & E, zinc, and selenium levels) of HIV- and HIV+ people in Ghana.

We examined the relationship between AFB₁ albumin adducts (AF-ALB) in plasma and the AFM₁ metabolite in urine and plasma concentrations of retinol (vitamin A) and α -tocopherol (vitamin E) in Ghanaians. We also measured aflatoxin B1 albumin (AF-ALB) adduct levels and vitamins A and E concentrations in the plasma of HIV-positive and HIV negative Ghanaians and examined the association of vitamins A and E with HIV status, aflatoxin levels and hepatitis B virus (HBV) infection. In 2010 we published a paper on vitamins A and E levels in HIV negative people and in 2011 we published one on HIV positive people (*Obuseh, F., Jolly, P.E., Jiang, Y., Shuaib, F., Waterbor, J., Ellis, W.O., Piyathilake, C., Ellis W.O., Desmond, R.A., Afriyie-Gyawu, E., and Phillips, T. Relationship between aflatoxin B1 albumin adducts in plasma, aflatoxin M1 in urine and vitamin A and E levels in Ghanaians. International Journal of Vitamin and Nutrition Research 80:355-368, 2010 and Obuseh, F., Jolly, P.E., Preko P.O., Kulczycki A., Ehiri J., Waterbor J., Jiang, Y., Piyathilake C.J. Desmond R.A. Aflatoxin exposure and health characteristics associated with vitamin A and E levels in HIV positive Ghanaians. Journal of the International AIDS Society, 2011, 14:53 doi:10.1186/1758-2652-14-53*). We have not been able to obtain funds or collaboration to conduct zinc and selenium tests. In the first paper we found that participants with high AF-ALB (≥ 0.80 pmol/mg albumin) had significantly lower vitamin A concentrations and marginally lower vitamin E. Conversely, AFM₁ was positively associated with vitamin and vitamin E. Participants with high AF-ALB or high AFM₁ (≥ 437.95 pg/dL creatinine) were almost 6 times more likely to be HBV. **These data indicate that aflatoxin may modify plasma micronutrient status resulting in vitamins A and E deficiencies.**

In the second paper we found that HIV-infected participants had significantly higher AF-ALB levels (median for HIV-positive and HIV-negative participants was 0.93 and 0.80 pmol/mg albumin, respectively; $p < 0.01$) and significantly lower levels of vitamin A (-16.94 $\mu\text{g/dL}$; $p < 0.0001$) and vitamin E (-0.22 mg/dL; $p < 0.001$). For the total study group, higher AF-ALB was associated with significantly lower vitamin A (-4.83 $\mu\text{g/dL}$ for every 0.1 pmol/mg increase in AF-ALB). HBV-infected people had significantly lower vitamin A (-5.66 $\mu\text{g/dL}$; $p = 0.01$). Vitamins A and E levels were inversely associated with HIV viral load ($p = 0.02$ for each), and low vitamin E was associated with lower CD4 counts ($p = 0.004$). **Our finding of the significant decrease in vitamin A associated with AF-ALB suggests that aflatoxin exposure significantly compromises the micronutrient status of people who are already facing overwhelming health problems, including HIV infection.**

Objective 4: Conduct cellular immune analyses using PBMCs from participants at recruitment and analyze data to identify immune impairments associated with aflatoxin biomarker levels in HIV+ individuals (compare HIV- with HIV+ participants). Examine interaction between HIV and aflatoxin on immune status.

We have been conducted immune analyses using PBMCs collected from the 300 HIV positive participants at recruitment and at the 12-16 months follow-up period. We are arranging the flow cytometry data and will conduct the statistical analyses for the association between AF-ALB levels

and cellular immune status. This should identify and confirm immune impairments associated with AF-ALB levels in HIV positive individuals. Previously we found immune impairments such as lower percentages of perforin-expressing CD8+T cells and CD4+ T regulatory cells associated with AF-ALB levels in HIV infected individuals (*Jiang Y., Jolly, P.E., Preko, P., Baidoo, J., Wang, J-S., Ellis, W.O., and Williams, J.H. Aflatoxin related immune dysfunction in Health and in Human immunodeficiency virus diseases. Clinical and Developmental Immunology, 2008, Article ID 790309, doi:10.1155/2008/790309*). We will determine whether these findings are confirmed in this new study sample.

Objective 5: Conduct a follow-up study and examine the association between aflatoxin biomarker levels and progression of HIV disease to AIDS (CD4 count, HIV viral load, types of opportunistic infections and HIV stage). The AF-ALB and detailed clinical data outlined under objective 2 above will be analyzed to determine the association between AF-ALB exposure and HIV disease progression in the participants.

Objective 6: Determine the association between aflatoxin biomarker levels and the occurrence of active tuberculosis infection in HIV+ people in Ghana. This work has been completed and a paper published (*Keenan, J., Jolly, P.E., Preko, P., Baidoo, J., Jiang, Y., McGwin, G. Jr. Association between aflatoxin B1 albumin adduct levels and tuberculosis infection among HIV+ Ghanaians. Archives of Clinical Microbiology, 2011, Vol. 2, No. 3:3, doi:10.3823/230*). Aflatoxin exposure has been shown to cause cell-mediated immune suppression and enhance HIV viral replication. Such immune suppression from aflatoxin can impair resistance to acute and chronic infections. We found that Hazard Ratios (HRs) were significantly higher for developing symptomatic TB (HR 3.30, 95% CI 1.34-8.11) for those in the highest AF-ALB quartile compared to the lowest quartile. Significantly higher HRs were not observed for other infections investigated such as malaria, HBV, or pneumonia. Thus, those with the highest levels AF-ALB in their blood from dietary intake were found to have an increased hazard of symptomatic TB.

Objective 7: Determine the association between AF-ALB levels and health status (malaria, HBV, intestinal helminth infections, Hb, folate, vitamin A, vitamin E) and anemia in pregnant women and on birth outcomes (low birth weight, pre-term delivery, small for gestational age, stillbirth) in Ghana.

This work has been completed and the findings published. A cross-sectional study of 785 pregnant women was conducted to investigate the association of anemia status and birth outcomes with AF-ALB levels. Data were collected on demographic characteristics (age, education, socio-economic status, residence, and type of toilet facilities), obstetric history for current and previous pregnancies (stillbirth, ectopic pregnancy, preterm delivery, and LBW), illnesses, and treatments during the current pregnancy. Obstetric information was obtained from the women's antenatal care (ANC) charts. ANC charts provided information on gestational age at first ANC visit (assessed by palpation or ultrasound), number of antenatal care visits, tetanus immunization, malaria prophylaxis, antihelminthic medication, illnesses, and treatment during pregnancy. A single blood sample was collected in EDTA by venipuncture for determination of AF-ALB, malaria antigen, hemoglobin and folate levels. Stool samples were obtained for determination of intestinal helminth infections (*Ascaris lumbricoides, Trichuris trichiura, Strongyloides stercoralis, Enterobius vermicularis* and hookworms). We have analyzed the data to determine associations between AF-ALB levels and anemia and AF-ALB levels and birth outcomes.

We found that 100% of the women had AF-ALB in their blood. With regard to **birth outcomes**, pregnant women with aflatoxin levels in the highest quartile were twice as likely to have low birth weight infants when compared to women in the lowest quartile, there was a trend of increasing risk for low birth weight with increasing AF-ALB levels ($P_{\text{trend}}=0.007$). This association remained after *adjusting* for known confounders, including malaria parasitemia, anemia and worm infections.

With regard to **aflatoxin and anemia**, the odds of women being anemic increased 21% with each quartile of AF-ALB reaching an 85% increased odds in the “very high” compared with the “low” category. This association was stronger among women with malaria and findings were robust when women with evidence of iron deficiency anemia were excluded. **This study found a strong, consistent association between anemia in pregnancy and aflatoxin and suggests that the prevalence of anemia among these pregnant women is associated with AF-ALB levels in their blood. This is the first study to report this finding** which has practical implications for policy makers in developing countries to put in place documented methods to reduce aflatoxin exposure of their populations. This may contribute to achievement of Millennium Development Goals #s 4 & 5 which relate to reduction of infant and maternal mortality, respectively. *A review paper and 2 original research papers have been published from this work and a fourth paper is in press in Ghana Medical Journal.*