REDUCING THE RISKS OF AFLATOXIN THROUGH PUBLIC HEALTH INTERVENTIONS

by

Pornsri Khlangwiset

BSc., Mahidol University, Thailand, 1996

MPH, University of Pittsburgh, 2007

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This dissertation was presented

by

Pornsri Khlangwiset

It was defended on

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and approved by

Aaron Barchowsky, Ph.D., Professor, Department of Environmental and Occupational Health, Graduate School of Public Health, University of Pittsburgh

Claudette St. Croix, Assistant Professor, Department of Environmental and Occupational Health, Graduate School of Public Health, University of Pittsburgh

John Mendeloff, Professor, Graduate School of Public and International Affairs, University of Pittsburgh

Dissertation Advisor: Felicia Wu, Ph.D., Assistant Professor, Department of Environmental and Occupational Health, Graduate School of Public Health, University of Pittsburgh

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Pornsri Khlangwiset, PhD

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Aflatoxin, produced by the fungi *Aspergillus flavus* and *A. parasiticus*, is the most potent naturally occurring human hepatocarcinogen. Food crops colonized by these fungi, especially maize and groundnut, are the major sources of dietary aflatoxin exposure. Aflatoxin and chronic hepatitis B virus (HBV) infection, two liver cancer risk factors that synergize with each other, are prominent in sub-Saharan Africa and certain parts of Asia. Furthermore, increasing evidence from epidemiological studies suggests that aflatoxin may cause child growth impairment, which can increase risks of premature deaths. A broad range of aflatoxin control strategies, developed to reduce aflatoxin exposure or its toxicity, include preharvest, postharvest, and dietary interventions; as well as the HBV vaccine, which does not reduce aflatoxin exposure but reduces the risk of aflatoxin-induced liver cancer.

We compared the efficacy and the cost-effectiveness of four aflatoxin risk-reduction strategies: HBV vaccine, biocontrol (preharvest), a postharvest intervention package, and NovaSil clay (dietary) in preventing liver cancer and stunting in Nigeria. Aflatoxin and chronic HBV infection are attributable for 8-27%, and 59-62%, respectively, of total liver cancers in Nigeria. We found that the HBV vaccine provides the greatest health-based efficacy and the lowest cost to avert one disability-adjusted life year (DALY) in Nigeria, compared with the selected aflatoxin control interventions. The prospective burden of aflatoxin-related stunting in Nigeria varies depending on aflatoxin exposure levels, which can vary substantially by year and location. At higher levels of aflatoxin exposure, the burden of aflatoxin-associated stunting is significant. Preventing stunting by any of these interventions would greatly reduce the cost per DALY and turn these interventions from non-cost-effective to very cost-effective. Our technical feasibility assessments of these four interventions suggest some advantages and disadvantages of

each intervention over the others. These data are crucial components in a decision making process to effectively allocate public health resources, and to position interventions for further development of public health interventions to prevent some aflatoxin-related public health problems, especially in high risk populations.

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PREFACE

I always believe that behind one's success is a bunch of helping hands. Therefore, this part is devoted to them. First and foremost, I would like to express my gratitude for having such the wonderful people in my life here. One of them is my advisor, Dr. Felicia Wu. I thank her for her generosity, support, and patience. She is one of my role models for her devotion to develop a knowledge base in the mycotoxins field. Her continuous support was so tremendous. Without the support from Dr. Wu and my former advisors and mentor, Dr. Nancy Sussman, Dr. Jim Petersen, and Dr. Linda Pearce, I might not have been this far.

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1.0 AFLATOXINS

1.1 DISCOVERY OF AFLATOXIN

Aflatoxins are toxic metabolites produced by certain fungi. In 1960, over 100,000 turkeys in England died within a few months. This disease was first described as turkey X syndrome. Later it spread to ducklings and pheasants. Finally, after an intensive investigation, it was found that the reason of this epidemic in the poultry was caused by Brazilian peanut meal used to feed these poultry. Because of the course of the disease, fungi were suggested as the origin of these toxins(1).

The toxin is named aflatoxin since it is produced by a fungus; *Aspergillus flavus*. At present, it has been found that other fungi, including some strains of *A. parasiticus* , plus related species, *A. nomius* and *A. niger*, can produce aflatoxins. There are at least 6 types of aflatoxins identified according to their structure: B_1, B_2, G_1,G_2, B_{2A} and G_{2A} (1, 2). In addition, two metabolites, aflatoxin M_1 and M_2 have been found in the milk of the animals fed with aflatoxin contaminated food. B_1 and B_2 got B as their designation from their properties in producing blue fluorescence with UV light; whereas, G_1 and G_2 give yellow-green fluorescence under UV light. These toxins have closely similar structures. B_2, and G_2 are considered as dihydroxy derivatives of B_1, and G_1; M_1, and M_2 are 4-hydrogenated derivatives of B_1, and B_2, respectively(1). Aflatoxin B_{2A} and G_{2A} have been produced in a small amount by *A. flavus* and *A. parasiticus*. Moreover, *A. flavus* also produces other closely related compounds; aflatoxin GM_1, parasiticol and aflatoxicol (3).

Aflatoxins are highly contaminated in maize, peanuts, and oil seeds, such as cottonseed, but they are also detected in milk, cheese, tree nuts, almonds, figs, spices, and a variety of other foods and feeds (1). Poor storage conditions, especially during rainy seasons, can increase concentration of aflatoxins (4). Moreover, aflatoxins have been identified not only in raw

agricultural products, but also in processed foods because they are stable in most food processes (1). Humans are affected by consuming food contaminated with aflatoxins. (5).

Acute exposure to aflatoxins could lead to a condition called “aflatoxicosis”. Early symptoms of aflatoxicosis are anorexia, malaise, and mild fever. If individuals are acutely exposed to high levels of aflatoxins, the symptoms can progress to lethal hepatitis with vomiting, abdominal pain, jaundice, fulminant hepatic failure, and death. The fatality rate from aflatoxicosis is about 25% (6). Several factors, including environment, exposure level, and duration of exposure, age, and health status can influence the aflatoxin toxicity. One major epidemic of aflatoxicosis occurred in more than 150 villages in India in 1974. This outbreak caused 397 ill-persons and 108 deaths. The contaminated maize with aflatoxin levels of 0.25 to 15 mg/kg was identified. The minimum estimated daily intake of aflatoxins was about 55 ug/kgBW. Eight years later, another outbreak took place in Kenya. Twelve out of twenty people admitted to the hospital died in this event with a minimum aflatoxin dose of 38 ug/kgBW per day (5).

During another outbreak of aflatoxicosis in eastern Kenya in 2004-2005, 125 deaths were reported. This outbreak was caused by poorly harvested and low quality storage conditioned maize. The average AFB_1 level in maize samples was 4,400 ppb− 220 times of Kenyan limit of aflatoxin in food. From January to June 2004, 317 patients sought hospitals with the symptoms of liver failure (7). The numbers of aflatoxicosis cases might be underestimated, since people who had mild forms of aflatoxicosis might not have gone to hospitals.

In 1988 AFB_1 was classified as a potent carcinogen in humans by the International Agency for Research on Cancer (IARC) (1). Hepatocellular carcinoma (HCC) has been related to aflatoxins, especially in the presence of hepatitis B virus (HBV) and hepatitis C virus (HCV) (3, 4). The other health effects from chronic exposure to aflatoxins might include immunologic suppression, impaired growth, and nutritional interference (1). Aflatoxins have been found to affect growth in children who are exposed to aflatoxins in their early life (3). Chronic toxicities of aflatoxin are often resulted from ingestions of low or moderate levels of aflatoxins (5).

Though susceptibility to acute and chronic toxicities of aflatoxin varies along the species, for most species, LD50−the dose that kills 50% of tested animals− of aflatoxin ranges from 0.5 to10 mg/kgBW (1). Because of aflatoxin toxicities, the US Food and Drug Administration (USFDA) does regulate the levels of aflatoxins in food and feed. No more than 20 ppb of

aflatoxins is allowed in food or commodities in the US trade. Allowable limit of AFM_1 in milk is less than 0.5 ppb (8).

1.2 AFLATOXIN AND LIVER CANCER

Being exposed to low levels of aflatoxins increases risk of liver cancer (7). However, some studies showed that a single, high doses of aflatoxin also induce liver cancer (9). Co-presence of aflatoxin and HBV increases the risk of liver cancer several times higher than either exposure alone. The proposed mechanisms of aflatoxin on liver cancer are (i) aflatoxins may suppress DNA repair mechanisms , which help to limit the progression of HBV infection to liver cancer, and (ii) HBV might interrupt the detoxification process of aflatoxins (10).

1.2.1 Mode of toxicity

It has been believed that the metabolic pathway of AFB_1 plays an important role in its toxicity, since metabolic activation is needed to exert its carcinogenicity (4). The main organ responsible for metabolism of aflatoxin is the liver; however, the metabolism of aflatoxin might occur at extrahepatic organs (11, 12). Metabolism of aflatoxins can be divided to 3 phases: bioactivation, conjugation, and deconjugation.

1.2.1.1 Bioactivation

The CYP450 enzyme system, mainly 1A2 and 3A4, metabolizes AFB_1 to the reactive metabolite; AFB1-8,9 -epoxide (AFBO) (4). Other predominant metabolites of AFB_1 by CYP 450 system are , aflatoxin Q1 (AFQ_1), aflatoxin P1 (AFP_1), which are considered less toxic than the parent form (AFB_1) (9). AFM_1 is one of the major AFB_1 metabolites. Though less mutagenic, AFM_1 is considered equally toxic as AFB_1 (13).

AFBO is highly unstable. It readily reacts with other cellular molecules. In the human liver, even both exo and endo isoforms of AFBO have been found, but the exo isoform is

predominant, much more mutagenic, and much more efficient to form DNA-adduct than the endo epoxide. At low substrate level, CYP1A2 is 3-6 times more efficient than CYP3A4 in metabolizing AFB_1 to AFBO, with the ratio between exo and endo isoform of 1:1. Even both AFM_1 and AFBO are produced by CYP1A2 activity, but the ratio between AFBO and AFM_1 produced is about 2.5:1(9).

CYP 3A4 can either metabolize AFB_1 to AFQ_1−non genotoxic metabolite−or to AFBO at the ratio of 10:1, but because of the high expression levels of CYP3A4, it is the major contributor to AFBO production (9). CYP3A5 is not found enormously in adult liver compared with CYP3A4, but a small percentage of population carries the functional variant termed CYP3A5*1 and can express higher or equivalent levels of CYP3A5 when compared to CYP3A4. Furthermore, since AFBO is preferentially produced by CYP3A5 under low substrate conditions, CYP3A5 acts as an bioactivation pathway, while CYP3A4 acts as a detoxification pathway (9).

All metabolites of AFB_1 are hydroxylated, but only AFM_1 is considered toxic via oral ingestion. However, this metabolite is detoxified by conjugation with taurocholic or glucuronic acid before excretion to the bile or urine (14).

Other non-CYP activating pathways of aflatoxins include epoxidation of AFB_1 from prostaglandin H synthase and DNA-bound derivative of AFB_1 by enzyme lipoxygenase (9).

1.2.1.2 Conjugation

Several enzymes involve in this phase including glutathione S-transferase (GST), ß-glucuronidase, and sulfate transferase which produce conjugates of AFB_1-glutathione, AFB_1-glucuronide, and AFB_1-sulfate, respectively. Conjugation makes the toxin more hydrophilic and readily to excrete in the bile. This is essential to reduce toxicity of aflatoxins (15). However, human cytosolic fractions poorly conjugate AFBO with glutathione (9), which results in the low ability of the body to excrete aflatoxin.

1.2.1.3 Deconjugation

This phase happens in intestinal tract by intestinal bacteria. This results in re-absorption of aflatoxin metabolites (16) or fecal excreted in an unbound form of aflatoxin(17). However, this phase has not been well characterized (16).

1.2.2 Pathways of pathogenesis of aflatoxin-induced liver cancer

Carcinogenic effects of AFB_1 are generally caused by the formation of AFB_1-DNA adduct, AFB_1-^{7}N-guanine, (AFB_1-N7-Gua) leading to the base transversion from guanine to thymine. AFB_1-N7-Gua is formed by covalently bonding C-8 of AFB1-8,9-exo-epoxide and ^{7}N-guanine base in DNA(15). AFB_1-N7-Gua are naturally converted to two secondary lesions, an apurinic site and a stable form–AFB_1-formamidopyrimidine (AFB_1-FAPY) adduct (4). The stable adduct is believed to be the most mutagenic lesion (4, 9).

There was a wealth of evidence that the carcinogenicity of aflatoxin is correlated well with the number of DNA-adducts (9). There has been evidence from the Kenyan aflatoxin outbreak in 2004-2005 showing that the levels of DNA adducts in aflatoxicosis survivors are 10 times higher than that of controls. People who died from this outbreak had more DNA adducts than the survivors (7).

A mutation of p53 at codon 249 is believed to be associated with aflatoxin exposure. P53 acts as a cancer suppressor gene by inducing apoptosis in damaged cells. In a normal situation, p53 binds to the protein upstream p21 and activates p21 transcription. P21 protein binds to cyclin dependent kinase 2 (cdk2) and impedes the roles of cdk2 in cell division. This process is an important checkpoint in DNA replication leading to repair before the next stage of cell divisions. Thus the cell division and proliferation are inhibited by the binding of p53 and p21. Therefore, a mutation in p53 leads to uncontrolled cell division and proliferation, which can later result in tumor formation. A mutation in p53 is believed to be the underlying mechanism of HCC development (18).

How aflatoxins induce liver cancer is complicated. Whereas the affinity of aflatoxins to other codons, such as codon 245 and 273, is similar or greater than its affinity to codon 249, only the codon 249 (AGGCC) represents a site of intermediate affinity for the AFB_1-induced lesion. Therefore, not only the affinity of aflatoxins to codons but also some factors not yet indentified influence the selectivity in the sites of mutations (4).

1.3 AFLATOXIN AND OTHER AFLATOXIN TOXICITIES

1.3.1 Immunosuppression

Most mycotoxins affect the immune system. Suppression of the immune system makes animals or humans more vulnerable to infectious agents. Several animals infectious outbreaks in the past were associated with the presences of mycotoxins. Salmonellosis and candidiasis outbreaks in domestic animals in 1960 were associated with Turkey X syndrome caused by aflatoxins. In 1977, following the presence of high concentration of aflatoxins in corn crops, a salmonellosis outbreak in swine took place in the Southeastern United States (19).

1.3.1.1 Animal studies

The effects of aflatoxins on the immune system have been reported in several animal studies. These effects include, but are not limited to, cell-mediated immune response suppression, lymphoblastogenesis suppression, impairment of delayed cutaneous hypersensitivity, impairment of the graft-versus-host reaction, the decreases in splenic CD4 (helper T) cell numbers and interleukin 2 (IL-2) production, and absence of the heat-stable serum factors involved in phagocytosis. Surprisingly, aflatoxin also affected parasite. Morbidity from malaria was decreased in mice treated with aflatoxins by the direct effect of aflatoxins to plasmodium parasite (10).

Aflatoxins impair macrophage function in the host defense system (10) and suppressed murine macrophages' productions of nitric oxide, superoxide anion, hydrogen peroxide, TNF-α, IL-1, and IL-6 (20). AFB_1 at ≥ 100 pg/ml is cytotoxic to monocytes. Even at the much lower doses as 0.5-1 pg/ml, aflatoxin could inhibit phagocytosis ability of monocytes to *Candida albicans* (10). Marin and colleagues found a decrease in the immune response induced by *Mycoplasma agalactiae* in weanling piglets exposed to 280 ppb aflatoxins. The mRNA expressions of proinflammatory IL-1β and TNF-α in aflatoxins treated weanling piglets (140 and 280 ppb aflatoxins) were decreased (21).

Several studies revealed that aflatoxins have more profound effect on cell mediated immune responses than humoral immunity (19); however, aflatoxin may reduce the efficiency of

vaccinations by decreasing humoral antibody responses to vaccines. This effect has been observed in poultry, rabbits, and dairy cattle. In a poultry study, aflatoxin reduced antibody titers to Newcastle disease, infectious bronchitis, and infectious bursal disease vaccines in the poultry treated with 200 ppb of aflatoxin for less than 40 weeks (10). Furthermore, immunosuppressive activities induced by aflatoxin can be transferred across the porcine placentas. The offspring of pigs and rats treated with aflatoxin contaminated diet exhibited the reductions in humoral immune functions (10).

1.3.1.2 Human studies

Recently, a study found that aflatoxins reduced phagocytosis in normal human peripheral monocyte *in vitro* (10). A survey conducted in Gambia, where the population has the highest record of chronic exposure to aflatoxin, showed the reverse relationship between the saliva immunoglobulin (sIgA) and AFB_1-albumin (AF-alb) adducts levels (22).

Jiang *et al.* (2005) determined the relationship between cellular immune response and AF-alb adducts levels in 64 Ghanaians. They found that the individuals with high AFB1-albumin AF-alb adducts levels had higher levels of CD69+ activation biomarker (CD3+69+ and CD19+69+) than participants who had low levels of AF-alb adducts (23). CD69+ is the molecule which seems to be the earliest inducible cell surface glycoprotein acquired during lymphoid activation (24). These activated T cells and B cells, (CD3+69+ and CD19+69+), are important for the body to respond against infectious diseases and for the productions of antibody to vaccines. Also, the CD8+T cells which contained perforin or both perforin and granzyme A, used in a cell killing process, were found in a lower percentage in the Ghanaians who had high levels of AF-alb adducts (20). However, the percentages of monocytes in peripheral blood are not affected by aflatoxins. Whereas, Jiang and colleagues found a non significant reduction in human macrophage phagocytosis in individuals having high AF-alb adducts, this effect is more prominent in several animal studies (20). The difference in the amplitude of responses might suggest the existence of the species differences in the immunomodulating effect induced by aflatoxins.

1.3.1.3 Interaction between aflatoxin and human immunodeficiency virus (HIV) infection

Recently, Jiang and colleagues reported an interaction between aflatoxin and acquired immune deficiency in Ghanaian population. One of the major findings from this study was that individuals with high AF-alb adducts (≥ 0.91 pmol/mg albumin) had lower percentages of CD4+ T regulatory cells and naïve CD4+ T cells and lower percentages of B-cells, compared with the HIV patients, who had low levels of AF-alb adducts (<0.91 pmol/mg albumin) (25). The interactions between aflatoxin and HIV infection, therefore, could exacerbate HIV status, accelerate progression to AIDS, and worsen quality of life. Williams et al. (2004*)* suggested that oxidative stress induced by aflatoxins could be an underlying interaction effect of aflatoxin and HIV, since oxidative stress increases HIV replication by direct effect or through the induction of infected leucocyte apoptosis(10).

1.3.1.4 Mechanism

The mechanism of immunosuppression-induced by mycotoxins is believed to be due to the inhibition of DNA, RNA, and protein syntheses. Aflatoxin may regulate the immune system through the modulation of cytokine production by a selective inhibition of certain mRNAs. Aflatoxins may inactivate some kinases, which inactivate genes coding for cytokines.

AFB_1 strikingly raised mRNA levels of major cytokines from macrophages, but suppressed the productions of their proteins. However, the same dose of AFB_1 slightly reduced mRNA and protein syntheses of cytokines from lymphocyte (26). These findings suggested that aflatoxins have predominant effect on m acrophages and affect protein transcription and translation in macrophage (20).

1.3.2 Growth impairments

Children could be exposed to aflatoxins during their early lives. Initially after birth, if mothers consume diets contaminated with aflatoxin, the infants are exposed to the hydroxylated metabolite of aflatoxin, aflatoxin M_1 (AFM_1), through breast milk. Later, they gradually make the transition from breast milk to other sources of nutrients. This is a dynamic process, in which

the transition involving first the combination of breast milk and weaning food, then family food, will completely replace breast milk (27).

According to Ghana survey in 1987, 58% of children had body weights below 80% of the National Center for Health Statistics (NCHS) weight-for-age, 8% were severely malnourished, and 52% were stunted. In Ondo State, Nigeria, a survey in 1986 revealed that 32% of children aged 6-36 months were stunted, 7% were wasted, and 28% were severely malnourished. A later national survey in 1990 showed that 43% and 22% of children were stunted and wasted, respectively (28).

There has been a wealth of evidence from either laboratory or epidemiological studies suggesting an association between aflatoxin and growth performances, of aflatoxin exposure in children (see chapter 5). Moreover, in *utero* aflatoxin exposure is associated with infant's low birth weights (29, 30), lower height at birth (31) and lower increases of the heights and weights of children within first year of life (32)

1.3.2.1 Mechanism

The mechanisms by which of aflatoxin leads to stunted growth in children have yet to be understood. Several possible mechanisms have been proposed, including immune suppression and intestinal toxicity. In animal studies, aflatoxins cause immunosuppressive effects either in the cell mediated immune system or the humoral immune system. Nontheless, the reduction in sIgA, an important component in mucosal immunity, was found in aflatoxin exposed children (22). The impairment in mucosal immune response makes the intestinal epithelium vulnerable to bacteria or toxins and could i ncrease local inflammation (27) thus impairing nutrient reabsorption.

2.0 COSTS AND EFFICACY OF PUBLIC HEALTH INTERVENTIONS TO REDUCE AFLATOXIN–INDUCED HUMAN DISEASE

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Pornsri Khlangwiset, Felicia Wu

Department of Environmental and Occupational Health, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA USA

2.1 ABSTRACT

This study reviews available information on the economics and efficacy of aflatoxin risk-reduction interventions, and provides an approach for analysis of the cost-effectiveness of public health interventions to reduce aflatoxin-induced human disease. Many strategies have been developed to reduce aflatoxin or its adverse effects in the body. However, a question that has been under-addressed is how likely these strategies will be adopted in the countries that need them most to improve public health. This study evaluates two aspects crucial to adoption of new technologies and methods: the costs and the efficacy of different strategies. First, we describe and categorize different aflatoxin risk-reduction strategies into preharvest, postharvest, dietary, and clinical settings. Then we compile and discuss relevant data on the costs and efficacy of each strategy, in reducing either aflatoxin in food or its metabolites in the body. In addition, we describe which crops are affected by each intervention, who is likely to pay for the control strategy, and who is likely to benefit. A framework is described for how to evaluate cost-effectiveness of strategies according to World Health Organization standards. Finally, we discuss which strategies are likely to be cost-effective and helpful under different conditions worldwide of regulations, local produce and soil ecology, and potential health emergencies.

2.2 INTRODUCTION

Aflatoxins are secondary metabolites of the fungi *Aspergillus flavus* and *A. parasiticus*. These species are prevalent in food crops – particularly maize, groundnuts, oilseeds, and tree nuts - in tropical and subtropical regions worldwide. Factors that influence whether these fungi produce aflatoxin include drought stress and rainfall, adaptation of crop genotype for its climate, insect damage, and agricultural practices. These fungi can also produce aflatoxin in postharvest conditions: storage, transportation, and food processing. Maize and groundnuts are the major sources of human exposure (the number of exposed persons exceeding several billion) because of these foods' high consumption rates worldwide and their susceptibility to aflatoxin contamination (6).

Aflatoxin B_1 (AFB_1), the most toxic aflatoxin, is the most potent naturally occurring chemical liver carcinogen known. For people who are chronically infected with hepatitis B virus (HBV; common in China and Africa), aflatoxin consumption raises by up to thirty-fold the risk of hepatocellular carcinoma (HCC; liver cancer) compared with either exposure alone (33). Acute aflatoxicosis, characterized by hemorrhage, acute liver damage, edema, and death, can result from extremely high doses of aflatoxin. In recent years, hundreds of aflatoxicosis cases in Africa have been associated with consumption of contaminated home-grown maize (34). Aflatoxin exposure is also associated with immunotoxicity in humans (10, 22, 23, 25), and with stunted growth in children (32, 35, 36).

To limit aflatoxin exposure, over 100 nations worldwide have set maximum tolerated levels (MTLs) of aflatoxin in food (19). These standards offer public health protection in industrialized nations, but arguably have little effect in less developed countries (LDCs), for several reasons. First, the food consumed from subsistence farms, which are widespread in LDCs, rarely enters any sort of regulatory inspection for aflatoxin (6, 10). Second, even if this food did meet the MTLs for aflatoxin, many people in LDCs consume such high levels of maize and groundnut products that their daily aflatoxin exposure would still render them vulnerable to disease (37). Third, LDCs that attempt to export maize and nuts abroad may find their export markets severely jeopardized by strict aflatoxin standards, resulting in potential countervailing risks of exporting the best foods and keeping the worst domestically (38).

Hence, it is estimated that about 5 billion people worldwide suffer from uncontrolled exposure to aflatoxin (6). Aflatoxin-associated health effects pervade sub-Saharan Africa and East Asia. These effects could be mitigated through effective use of current agricultural knowledge and public health practice. The discussion of this problem and its remedies must include the underlying question of food insufficiency and more general economic challenges in developing countries (6).

Several interventions to reduce and prevent aflatoxin toxicity have been developed. These range from aflatoxin control methods in agricultural practice through chemopreventive dietary constituents to vaccination against HBV. Agricultural interventions to reduce aflatoxin could be done either in preharvest (field) or postharvest (drying, storage, transportation, etc.) conditions. Meanwhile, there is growing research interest in using certain substances available in foods and natural products to reduce aflatoxin's adverse impacts in the body. By binding

aflatoxin in the gastrointestinal tract, or inducing enzymes involved in aflatoxin metabolism pathways, several substances can reduce aflatoxin bioavailability in humans. The HBV vaccine neither reduces aflatoxin levels in food nor reduces aflatoxin's bioavailability in the body; however, it reduces aflatoxin-induced liver cancer by greatly reducing the risk of chronic HBV infection, thereby preventing the synergistic impact of HBV and aflatoxin in HCC pathogenesis.

Understanding the costs and efficacy of different aflatoxin control interventions can help decision makers–be they government policymakers or farmers or consumers–to optimally allocate resources, particularly in conditions of scarcity. Wu et al. (2008) presented three case studies for cost-effectiveness of aflatoxin control in the United States: two biocontrol agents (Afla–Guard^{TM} in groundnuts and AF36 in cottonseed) and a transgenic crop (Bt maize) (39). However, this assessment was limited to the US, and may not have equal applicability worldwide. This study reviews the available data on costs and effectiveness for interventions that could be used to control aflatoxin from a global perspective.

Table 2-1 shows the factors that we include in our analysis. First, we are interested in whether the intervention is agricultural (methods that take place in the field or postharvest settings), dietary (supplements or processing or natural constituents in food), or clinical (HBV vaccination). This gives us information about who needs to implement the intervention, and how often and in what context it needs to be done. Second, we are interested in whether the intervention in question reduces aflatoxin concentrations in food, or whether it reduces bioavailability of aflatoxin or its metabolites in the body. This provides useful information on the nature of the intervention and whether the intervention can potentially reduce health impacts, trade losses, or both. Third, we are interested in how much the intervention costs, and how effective it is. These are obviously the main factors in a cost-effectiveness assessment. Finally, we are interested in who pays for the intervention (e.g., growers, consumers, or local / national government) and who benefits from it.

Table 2-1. Factors included in cost-effectiveness analysis of public health interventions to reduce aflatoxin and its related illnesses.

Factor	Categories	Rationale
Stage at which intervention occurs	• Agricultural • Dietary • Clinical	To understand how many people, and what group of people, must implement the intervention; and under what conditions
What the intervention reduces	• Aflatoxin levels in food • Bioavailability of aflatoxin and its metabolites in body	To determine whether the intervention reduces adverse health effects, adverse market effects, or both
Cost–effectiveness of intervention	• Cost • % reduction of aflatoxin or bioavailability of aflatoxin / metabolites	To determine the economic factors underlying each intervention: costs vs. potential benefits
Stakeholder involvement	• Who pays for the intervention • Who benefits from the intervention	To understand if the appropriate economic and health incentives exist for people to adopt the intervention

2.2.1 Preharvest interventions

Because most aflatoxin problems begin and develop in the field, strategies are needed to prevent infection of growing plants by toxigenic fungi. Developing genetic resistance to Aspergilli in maize and groundnuts is a high priority (40, 41). Worldwide, the advantages of using resistant plant genotypes include direct health and economic benefits, the lack of impact on crops or the environment, and the ability to use these genotypes in combination with other aflatoxin control strategies (42).

A number of resistant inbred maize lines have been indentified, including MI82 (43), Mp420, Mp313E, and GT-MAS:gk (44). Sources of resistance to each of these pathogens have been identified and have been incorporated into public and private breeding programs, and have

been extended to include germplasm lines from Africa (42, 45). Potential biochemical and genetic resistance markers have been identified in crops, particularly in maize, which are being utilized as selectable markers in breeding for resistance to aflatoxin contamination (40). Several proteins associated with resistance (RAPs) include, but are not limited to, globulin-2 proteins, late embryogenesis abundant proteins (LEA3 and LEA14), a stress-related peroxiredoxin antioxidant (PER1), heat-shock proteins (HSP17.2), a cold-regulated protein (COR), and an antifungal trypsin-inhibitor protein (TI) (46). Now that the sequencing of the *A. flavus* genome has been completed, and genes that potentially encode for enzymes involved in aflatoxin production have been identified, genomics as a tool for combating aflatoxin biosynthesis has gained much ground (47-49).

The development of groundnut cultivars with resistance to preharvest aflatoxin contamination has also yielded promising results. Screening techniques have been developed that can measure genetic differences in susceptibility to aflatoxin contamination, and these techniques have been used to identify multiple accessions that have shown significant aflatoxin reduction in multiple environments. Groundnut genotypes with drought resistance have also shown aflatoxin reduction (50, 51). Aflatoxin resistant genotypes have been developed in other parts of the world, and have shown success in aflatoxin reduction (52).

Transgenic (genetically modified) crops may also play a role in reduction of preharvest aflatoxin accumulation. Insect damage is one factor that predisposes maize to mycotoxin contamination, because insect herbivory creates kernel wounds that encourage fungal colonization, and insects themselves serve as vectors of fungal spores (53, 54). Bt maize is one of the most commonly grown transgenic crops in the world today. It contains a gene from the soil bacterium *Bacillus thuringiensis* (hence the name Bt), which encodes for crystalline proteins that are toxic to certain members of the insect order Lepidoptera (reviewed by Wu 2007). Earlier Bt events showed only mixed success in controlling aflatoxin (55), as they provide insect protection primarily against European corn borer and Southwestern corn borer, as opposed to the insects that have been associated with aflatoxin contamination: fall armyworm and maize earworm. However, a new Bt event that has just become available commercially and provides enhanced protection against these insects has shown promise in significantly reducing aflatoxin in field trials (56). In addition to Bt maize, prototypes of genetically engineered crops have been developed that contain genes encoding fungal growth inhibitors for reducing fungal infection.

Gene clusters housing the genes governing formation of aflatoxin have been elucidated and are being targeted in strategies to interrupt its biosynthesis (40).

Biocontrol of aflatoxin refers to the use of organisms to reduce the incidence of Aspergilli in susceptible crops, so as to reduce aflatoxin contamination. The most widely used biocontrol method employs atoxigenic strains of Aspergilli that can competitively exclude toxigenic strains from colonizing crops. These biocontrol methods have been used in maize, groundnuts, and cottonseed worldwide (57-62). Importantly, atoxigenic *A. flavus* strains have been found in sub-Saharan Africa, which show promise for controlling aflatoxin in African maize (59, 62). Biocontrol methods, though applied in the field, can result in reduced aflatoxin in crops for as long as six months postharvest (Dr. Ranajit Bandyopadhyay, personal communication).

Cultural practices, including crop rotation, tillage, planting date, and management of irrigation and fertilization, can also help to prevent *Aspergillus* infection and subsequent aflatoxin accumulation by reducing plant stress. These practices can have important effects on infection and subsequent mycotoxin accumulation (41). Ultimately, a combination of preharvest strategies, as described above, may be needed to adequately prevent mycotoxin contamination in the field (40).

2.2.2 Postharvest interventions

Current food storage and processing practices in industrial nations can prevent postharvest development of mycotoxins, but postharvest aflatoxin accumulation remains a threat in less developed countries (LDCs), especially in tropical areas. Hence, knowledge of the key critical control points during harvesting, drying and storage stages in the cereal production chain are essential in developing effective prevention strategies post-harvest (63). Possible intervention strategies include good agricultural and storage practices, such as early harvesting, proper drying, sanitation, proper storage, and insect management, among others (64). This is true not just for maize and groundnuts (the major sources of aflatoxin exposure for humans), but also for tree nuts such as pistachios, where there have been dramatic improvements in aflatoxin reduction in Iran due to improved drying and storage conditions over the past decade (65).

Removing existing aflatoxin contamination is possible by sorting aflatoxin-contaminated kernels from relatively cleaner ones. This can be done by either simple physical (e.g., handsorting) or flotation and density segregation methods. Sorting by these types of methods has been shown to significantly decrease aflatoxin levels in postharvest maize (66).

After sorting, there are several methods to prevent the growth of Aspergilli and hence reduce aflatoxin contamination postharvest. These include control of moisture levels in stored crops, temperature, and insect pests and rodents (66).

Combinations of these methods to reduce postharvest aflatoxin have been tested for efficacy in actual rural village conditions. Turner et al. (2005) describe a postharvest intervention package to reduce aflatoxin in groundnuts, tested in Guinea. The package consisted of six components: education on hand-sorting nuts, natural-fiber mats for drying the nuts, education on proper sun drying, natural-fiber bags for storage, wooden pallets on which to store bags, and insecticides applied on the floor of the storage facility under the wooden pallets (67).

In industrial nations, drying with forced air and supplemental heat is common to control moisture levels in crops. At 70°C, *A. flavus* infection in maize is significantly reduced compared to that in the maize dried at 40°C. But this method can potentially reduce seed germination and increase stress cracks (68).

Chemical methods can detoxify aflatoxins by reduction, destruction, or inactivation. These methods include ammoniation, acid treatment, oxidizing agents, and reducing agents; and are reviewed in-depth in Kabak et al. (2006). There are several issues and risks associated with these methods: it is difficult to detoxify the aflatoxin without reducing nutritive value and palatability; parameters such as reaction time, temperature, and moisture must be monitored; some necessary additional cleaning treatments can be expensive and time-consuming, and toxic byproducts may be produced.

2.2.3 Dietary and food processing interventions

A variety of dietary interventions can reduce aflatoxin-related health risks. One simple dietary intervention, where feasible, is to consume less maize and groundnuts, in favor of other food crops that have significantly lower aflatoxin contamination, such as sorghum and pearl millet

(69). Where it is not easy to make such a dietary shift, however (e.g., where maize and groundnuts have traditionally been staples), other dietary interventions may prove helpful.

One class of dietary interventions involves adsorption of aflatoxin. A dsorbent compounds can be included in food or feed or taken separately during mealtimes to bind aflatoxin in the gastrointestinal (GI) tract, resulting in reduced aflatoxin bioavailability. Several materials have varying degrees of this ability to bind aflatoxin, including bentonites, zeolites, diatomaceous earth, activated charcoal, and fibers from plant sources. One material that has proven effective in animal feed and is showing promise in human trials is calcium montmorillonite, marketed as NovaSil clay (NS). NS has been shown to prevent aflatoxicosis in many animal species when included in their diet, by binding aflatoxin with high affinity and high capacity in the GI tract (70). NS has been shown to reduce aflatoxin toxicity on body and organ weights, feed intake, and hepatic vitamin A when tested in broiler chicks. No toxicity has been found in a dose as high as 0.5% w/w in the diet (71). Phase I (72) and Phase II (73) clinical trials confirm the safety of NS for use in human food, and provide assurance that NS does not bind with vitamin A and E, thereby does not result in elimination of these nutrients.

Green tea polyphenols (GTPs) have been shown to inhibit chemically-induced cancers in animal and epidemiological studies (70, 74). GTPs inhibit initiation of aflatoxin-induced HCC in rats by modulating aflatoxin metabolism (75); and in humans, there are inverse associations between green tea consumption and cancer risk (76).

Chlorophyllin, a derivative of chlorophyll, is a natural constituent of green vegetables in the human diet that has shown anticarcinogenic effects in animals (77). Chlorophyllin appears to protect against aflatoxin by sequestering aflatoxin during the digestive process and hence impeding aflatoxin's absorption. In addition, chlorophyllin may have enzyme-inducing properties that contribute to its mechanism of detoxification (74, 78). Aside from binding aflatoxin, chlorophyllin is capable of binding certain carcinogenic substances, such as polycyclic aromatic hydrocarbon (PAH), heterocyclic amines, and other hydrophobic molecules (79). Moreover, other modes of action of chlorophyllin, such as scavenging free radicals (80), inducing apoptosis in cancer cells (81), inducing cell-cycle arrest, and altering markers of cell differentiation (82), have been proposed for its protective effects against DNA damage and colon cancer. Side effects of chlorophyllin are rare, but may include diarrhea and discoloration in urine and feces (83).

A variety of substances have the potential to reduce aflatoxin-induced HCC by inducing enzymes, such as glutathione-S-transferases (GSTs), that mediate conjugation of the reactive intermediate aflatoxin-8,9-epoxide. Genetic differences exist in the extent to which aflatoxin in the diet is biotransformed into this harmful epoxide; therefore, agents that induce GSTs have varying effectiveness among individuals. Dithiolethiones (oltipraz) and sulforaphane have this ability, and may also inhibit HBV transcription through elevation of p53 tumor suppressor genes (84). Oltipraz is an antischistosomal drug; while the precursors to sulforaphane, glucosinolates, can be found in cruciferous vegetables such as broccoli (74, 85, 86).

There is increasing evidence that some lactic acid bacteria have the ability to bind aflatoxin B_1 (87-89). These bacteria are important in the fermentation of many foods, including vegetables, fruits, and dairy products. The main purpose of *Lactobacillus* inclusion in food has typically been fermentation, not the prevention of aflatoxin risk. Hence, inclusion of culturally appropriate fermented foods in the diet may be a feasible method of partially reducing aflatoxin risk. Other methods of food processing have moderate ability to reduce aflatoxin and other mycotoxins (90), such as extrusion processing at temperatures greater than 150°C.

2.2.4 Hepatitis B vaccination

A regular practice now in the US and other developed nations, HBV vaccination in children is still rare in many parts of the world. Vaccinating children against HBV has been shown, over the last three decades, to significantly decrease HBV infection in several regions including Europe (91, 92), Taiwan (93), and Thailand (94). This vaccine has already had, and will continue to have, significant impacts on liver cancer incidence, particularly in Africa and East Asia, considering that roughly 65 million out of 360 million individuals who are chronically infected live in Africa (95). Though the vaccine itself has no impact on actual aflatoxin levels in diets, it reduces aflatoxin-induced HCC by lowering HBV risk, thereby preventing the synergistic impact of HBV and aflatoxin in inducing liver cancer. However, other health effects of aflatoxin such as retardation of growth and immunomodulation are not altered by HBV vaccination. Moreover, those who already have chronic HBV infection would not benefit from

the vaccine, which is why it is very important for this vulnerable subpopulation to avoid aflatoxin exposure as much as possible.

Table 2-2. Costs and efficacy of agricultural interventions to reduce aflatoxin and its adverse health effects.

Intervention		Efficacy		Cost	Who pays	Who benefits
		Aflatoxin reduction[a]	Aflatoxin adduct reduction			
Agricultural (preharvest and postharvest)	Aflatoxin resistance breeding (conventional and transgenic)	Conventional: >70%; 82–93% [G] (50, 52)	–	Research & development costs; no expected additional cost to grower	Institutes funding research	Growers, Consumers
		Transgenic: 47% in Bt maize (56)	–	$21/acre [Bt maize] (39)	Growers	Growers, Consumers
	Biocontrol using competitive fungi	60–87% [M] (58)	–	$10–$12/ha ($4–$5/acre; Dr. Ranajit Bandyopadhyay, personal communication) ¢0.52 –¢0.63/kg (Nigeria)	Growers	Growers, Consumers
		70–91% [G] (96)	–	$17–$32/acre [Afla-Guard™] (39) ¢0.21–¢0.39/kg (USA)	Growers	Growers, Consumers
		80% AF36 [C] (97)	–	$6–$16/acre [AF36] (39) ¢1.50–¢4.00/kg USA)	Growers	Growers, Consumers

Table 2-2 (continued)

Intervention		Efficacy		Cost	Who pays	Who benefits
		Aflatoxin reduction[a]	Aflatoxin adduct reduction			
	Irrigation+ insecticide	99% [M] (98)	–	$1,100–$1,300/acre to install irrigation (99) *Drip-Micro Irrigation System* $960–1,770 / acre for the drip on orchard (surface) irrigation system (CI) $1,300–2,250/acre for the drip on orchard (buried) irrigation system (CI) $640–1,600/acre (CI) + $240–480 (AC for hose replacement cost) for the above ground row crop drip system $ 1,440–4,010/acre for permanent installation cost of subsurface row crop drip (100)	Government, growers	Growers, Consumers
				Sprinkler Irrigation System $ 740–940/acre (CI)+ $130 –170 (AC) (101)		

Table 2-2 (continued)

Intervention		Efficacy		Cost	Who pays	Who benefits
		Aflatoxin reduction[a]	Aflatoxin adduct reduction			
				Surface Irrigation System $400–810/acre (CI) + $220/acre (AC) for the siphon tube $210–420/acre (CI) + $170/acre (AC) for the gated pipe $600–1,200/acre (CI) + $250 (AC) for the surge flow $340–680/acre (CI) + $190 (AC) for the cablegation pipe (102)		
	Postharvest intervention package (natural fiber bags, wooden drying pallets, insecticide)	69% [G] (67)	57.2% lower aflatoxin albumin adducts in humans	$61 per household for several years [bags and wooden pallets can be reused] (67)	Growers	Growers, Consumers
	Artificial drying			4.5 cents per bushel (M) (103)	Growers	Consumers

Note: **All cost data are converted to USD 2009 values. [a] C= cottonseed, M= Maize, G = Groundnut, NA = Not available, CI = Capital investment, AC = Annual cost

Table 2-3. Costs and efficacy of dietary and clinical interventions to reduce aflatoxin's adverse health effects.

Intervention		Efficacy		Cost	Who pays	Who benefits
		Aflatoxin adduct reduction	HCC reduction			
Dietary/ Chemo–prevention	NovaSil™ clay	58.7% lower AFM_1; ~25% lower Aflatoxin albumin adducts in humans (104)	–	$0.73 per person per year based on 3-g daily dose clay preparation (Dr. Timothy Phillips, personal communication 2009)	Consumers, government	Consumers
	Green tea polyphenols (500–1000 mg)	~ 43% lower AFM_1 in humans; > 15% lower aflatoxin albumin adducts at 500 mg dose (105); 20–30% lower AFB_1–DNA adduct in rats (75)	Up to 70% lower hepatic preneoplastic lesions in rats (75)	$0.20 − $1 per day (polyphenols levels range from 710.5 – 900 mg) (106-108)	Consumers	
	Chlorophyllin	55% lower AFB_1-N^7-Guanine in humans (78)	–	$0.10/day (Dr. Thomas Kensler, personal communication)	Consumers	
	Oltipraz	51% lower AFM_1 [500 mg weekly], 2.6-fold increase in aflatoxin B_1 mercapturic-acid excretion [125 mg daily] (109)	42% lower HCC incidence in F344 rats (110)	$ 59, $ 236 / per 5 and 25 mg (111) [Note: these are costs for analytical grade Oltipraz; no cost data for pharmaceutical grade]	Consumers	

Table 2-3 (continued)

Intervention		Efficacy		Cost	Who pays	Who benefits
		Aflatoxin adduct reduction	HCC reduction			
	Sulforaphane (400 µmol ~ 70 mg)	No significant reduction in AFB_1-N^7-Guanine, but inverse association for dithiocarbamate excretion and AFB_1-N^7-Guanine (85)	–	$24.80 for 30 capsules (0.21% of sulforaphane in 250 mg) (amazon.com); If consuming broccoli sprouts or sprouts tea: 1 dose (385 g sprouts) = $0.31	Consumers	
Vaccine	Hepatitis B vaccine	–	84–95% HBV reduction → 45–50% HCC reduction (112-114)	$910.41/death averted $23.09 per DALY averted (115)	Government	HBV-uninfected individuals

Note: ** All cost data are converted to USD 2009 values

2.2.5 Costs and efficacy of preharvest interventions

Breeding aflatoxin resistance into crops requires upfront research and development funds in and between various nations, depending on where the seed will be deployed. However, once the resistant strains of crops are bred, the seed need not be significantly more expensive to farmers than existing genotypes. Efficacy in reducing aflatoxin has been shown to be as high as 90–98% in resistant maize varieties developed and tested in the US (116). Groundnuts bred for aflatoxin resistance in the US achieved at least a 70% reduction in preharvest aflatoxin contamination in multiple environments (50). Similarly, naturally aflatoxin-resistant lines in India had significantly lower aflatoxin levels compared with susceptible lines and produced higher pod yield (52). These efficacies do not necessarily apply to maize- and groundnut-producing regions outside the US and India, but demonstrate what the breeding technologies have the potential to achieve. A caution with interpreting these differences in aflatoxin levels is that one should distinguish between naturally resistant vs. specifically bred lines in terms of aflatoxin reduction. Importantly, drought stress is a critical factor for preharvest aflatoxin contamination in groundnuts and maize. In years when drought stress is not critical, even susceptible lines of groundnuts are less likely to be contaminated by aflatoxin. Now breeding efforts are focused in releasing lines with the specific aflatoxin-resistant attribute rather than improving on serendipitous levels found in natural lines.

Transgenic maize varieties will likely incur a greater cost to growers; however, the cost of transgenic seed is lower in LDCs because biotechnology companies are providing free intellectual property there. In the US, Bt maize seed costs about $21 per acre more than conventional maize seed (39); the cost would be significantly lower in LDCs. Field trials of new Bt maize events in the US, which are effective against the insect pests that predispose maize to *Aspergillus* infection, show a 47% reduction in aflatoxin compared with non-Bt isolines (56).

The costs of biocontrol vary depending on the product and the locale. In the United States, the per acre cost of applying AF36 to control aflatoxin in cottonseed ranges from $6–$16 per acre, and achieves 70–90% aflatoxin reduction compared with cottonseed from untreated fields (39, 97). Afla-Guard™ applied to groundnuts costs about $17–$32 per acre (39), with 70–

91% aflatoxin reduction compared with untreated groundnuts (96). Because of the local strain-specific response and to avoid introducing foreign Aspergilli, it is important to identify local atoxigenic strains that potentially competitively exclude toxigenic strains. Biocontrol studies in Nigerian maize using local atoxigenic strains of *A. flavus* have shown efficacy levels at as high as 90%, with a cost of about $10–$12 per hectare: $4.04–$4.86 per acre (Dr. Ranajit Bandyopadhyay, personal communication).

Though the cost per unit area provides useful information for policy makers and growers to determine total cost for applying biocontrol to a specific area, the cost of biocontrol per consumption unit helps policy makers and growers to roughly screen whether biocontrol is economic feasible. To convert the cost data to consumption unit, we obtained production yields per unit area (117) and divided by the per unit area cost. In the U.S., predicted groundnuts production in 2009 (based on 2003–2007 FAOSTAT database) is about 3,300 kg per hectare, or 8,200 kg per acre, hence Afla-Guard™ costs 0.21–0.39 cents per kilogram of groundnuts. The cost of biocontrol per kilogram of maize in Nigeria ranges from 0.52 cents to 0.63 cents, based on the predicted maize yield in 2009 of 1,900 kg/ha.

A combination of irrigation systems and insecticide applications can reduce aflatoxin levels by 99% in maize, compared with non-irrigated, non-treated maize in the US (98). However, this combined intervention might be costly in LDCs where irrigation systems have yet to be installed widely. The cost of insecticide varies widely, depending on the locale and the chemical.

Methods for irrigation vary greatly from a simple method using watering-cans or buckets to complicated methods that require complex equipment and maintenance. In the US, the initial cost of drip- and micro-irrigation systems ranges from US $ 640 to $4,000 per acre, depending on the type; e.g. surface, buried or sub-surface drip systems (100). The capital cost per acre of sprinkler irrigation systems ranges from US $740–$940. The cost for ownership (depreciation, insurance, and interest) and operating per year is about US$ 130–$170 per acre (101).

Compared to sprinkler methods, surface irrigation systems, in general, need lower energy and capital requirements, but this method has disadvantages: higher labor requirements, lower water efficiency and potential soil erosion. The costs of four types of surface irrigation systems, including the siphon tube, the gated pipe, the surge flow, and the cablegation pipe have been estimated for 20 acres at $220, $170, $250, and $190, respectively (102). The estimated cost for

irrigation system installation is about $1,100–1,300 per acre (99), but if traditional crop-watering methods are used, the cost is much lower (and efficacy is perhaps also lower).

2.2.6 Costs and efficacy of postharvest interventions

Aflatoxin control can also be achieved by sorting, proper drying of food, and suitable storage conditions. Mechanical blanching and sorting of groundnuts in the US has the ability to almost completely eliminate aflatoxin, and the blanching and sorting each cost about $150–$170 per ton (118). In LDCs, it is far less common to have blanching and sorting machines, so most sorting of groundnuts is done by hand. The cost in terms of lost product varies enormously, depending on aflatoxin levels in any given harvest. Even the efficacy of sorting may vary, depending on how well farmers can identify aflatoxin-contaminated nuts – hence, the importance of education and outreach to farmers on aflatoxin contamination and its identification. However, time-related costs should also be taken into account. It is estimated that one farmer would require an entire day to hand-sort 40–50 kg groundnuts (Dr. Jonathan Williams, personal communication).

Turner et al.'s (2005) postharvest intervention package reduced aflatoxin levels in groundnuts by 69% compared with control groundnuts. Moreover, mean serum aflatoxin albumin adducts in villagers adopting this package was 57.2% lowered than that of the control villagers five months after harvest. While the initial cost of this package was about $50 per household in 2005 to improve the storage condition of 25 groundnut bags, many components of the package last for several years (e.g., the wooden drying pallets, storage bags, and insecticide) (67).

The cost of artificial drying to reduce aflatoxin depends on the costs of fuel and electricity, and the differences of moisture content (MC) in harvested crops and the required levels. Reducing one point of moisture from a bushel of maize (25.40 kg) costs about 4.5 cents (103). Whether they choose to dry their product using natural or artificial drying method, growers in developed countries, somehow, have to "pay" for excess moisture left in their grains. Increase in field loss due to stalk lodging, insect and spreading of ear molds often happens with field drying method. Grain storage operators charge growers about 11–12 cents per point (MC) per bushel (103) for drying grain delivered too wet.

One chemical treatment option, ozonation, costs only $5 per ton (119). Although ozonation can completely degrade AFB_1 at high moisture and temperature for 2 hours, animals fed with the treated cottonseed and peanut meal had lower protein efficiency ratios than those fed the aflatoxin-contaminated meal, indicating that ozonation might degrade essential nutrients or produce new toxins (119).

2.2.7 Cost-effectiveness of dietary aflatoxin risk reduction strategies

Dietary interventions do not directly reduce aflatoxin in food, so aflatoxin biomarkers are the important intermediate endpoints to measure the efficacies of diet against aflatoxins' toxicities. Several metabolites of AFB_1 and aflatoxin macromolecular adducts are currently used as biomarkers of aflatoxin exposure. The most commonly used biomarkers for recent (short-term) aflatoxin exposure are urinary aflatoxin M_1 (AFM_1), and aflatoxin DNA adducts. AFM_1, secreted in urine and breastmilk, is an oxidative metabolite of AFB_1. Levels of AFM_1 reflect aflatoxin exposure in the past 24 to 48 hours. In humans, it is estimated that about 0.2% of AFB_1 is excreted as AFB_1-N^7-Guanine (120). Twenty-four hour excretions of aflatoxin B_1-DNA adduct at N^7 of guanine (AFB_1-N^7-Gua) in urine following aflatoxin exposure in rats have shown to be linearly correlated with aflatoxin exposure. AFB_1-N^7-Gua, aside from being a biomarker for aflatoxin exposure in more short-term time scales (over the last day), reflects DNA damage that can in the long term increase risk of developing HCC (121). It is noteworthy that high variations of the short-term aflatoxin biomarkers, which may reflect the heterogeneity of contamination, have been reported (104, 105). An increased excretion of urinary aflatoxin mercapturic acid (AFB_1-NAC), a phase 2 AFB_1 metabolite, is reported in a study of oltipraz, which is believed to induce phase 2 enzyme metabolism (109).

Unlike urinary AFM_1 and AFB_1-N^7-Gua, albumin adducts of aflatoxin provide integrated levels of aflatoxin exposure over a period of months because of the relatively long turnover period of albumin (the half-life of albumin is approximately 20 days in healthy individuals). Though the longer period (e.g., years) of aflatoxin albumin adducts in body is also proposed (104). The level of aflatoxin albumin adducts in maternal blood has been associated with decreased height and weight gain during the first year of life (32).

Aside from the aforementioned biomarkers, radiolabeled aflatoxin B_1 has been used as markers in several in *vitro* and in *vivo* aflatoxin studies (122-124). The results from a chicken study, in which carbon-14 (^{14}C) radiolabeled aflatoxin was used as a marker, showed that with NovaSil doses of 0.5%, bioavailability of aflatoxin in the blood and liver were 5.3% and 14.6%, respectively, compared to those in the control group (124). Aflatoxin-albumin adducts in both low-dose and high-dose NovaSil intervention arms – when administered in capsules three times daily – were significantly lower than those in the control arm after 3 months, with a roughly 25% reduction: 0.89–0.90 pmol mg^{-1} vs. 1.20. A 58.7% reduction in AFM_1 was also observed in the high-dose arm three months into the study (104). Adding 4.5 kg of calcium montmorillonite clay to a ton of animal feedstuffs costs $2–$6 (125). Texas Enterosorbent, Inc. has developed a new related product intended for future human use: calcium aluminosilicate/uniform particle size NovaSil (CAS/UPSN). This new product would cost about 18 cents per 3-gram daily dose (Dr. Robert Carpenter, personal communication). If NovaSil were blended into food, such as maize meal, the cost could be decreased and the efficacy might be increased, depending on dose. As such, the cost could be as low as $0.73 per person per year (Dr. Timothy Phillips, personal communication).

Green tea polyphenols (GTPs) appeared to inhibit aflatoxin-induced initiation of HCC in rats, with 20–25% lower AFB_1-DNA adducts compared with rats in a control group (75). In a human study in China, subjects in an intervention group receiving 500 mg daily GTP had 13% lower aflatoxin-albumin adduct levels after 3 months, compared with the placebo group; but there was no significant difference in albumin adduct levels between the placebo group and a group that received 1000 mg daily GTP. There was, however, about a 43% lower AFM_1 level in both GTP intervention groups compared with the control group (105). There were no significant differences between the groups after 1 month. The cost of GTPs ranges enormously depending on how it is administered: in the form of green tea, or in capsules. For tea that can be purchased in retail outlets, costs range from $0.20–$1.00 per day (106-108), providing a range of GTPs from 710–900 mg.

In a randomized, double-blind, placebo-controlled trial in China, subjects who consumed chlorophyllin in each meal for 4 months showed 55% lower aflatoxin-N^7-guanine adducts compared with subjects in the control arm (78). The cost of chlorophyllin is comparable to that of NovaSil: about $0.10 per daily dose (Dr. Thomas Kensler, personal communication).

Oltipraz administered in different schedules at different doses results in varied changes in aflatoxin biomarkers, indicating alterations in both Phase 1 and 2 metabolism of aflatoxin. In a human intervention group in China, one month of 500 mg oltipraz administered weekly resulted in 51% lower AFM_1 (phase 1 metabolite) levels compared with a placebo group, but no difference in aflatoxin-mercapturic acid (phase 2 metabolite) levels was found. Lower doses of oltipraz (125 mg) administered daily resulted in a 2.6-fold increase in aflatoxin-mercapturic acid excretion, but no difference in AFM_1 (109). In a study involving rats administered high aflatoxin doses for 5 weeks, those given oltipraz during each week achieved a 42% reduction in HCC risk (110). No cost information is available on pharmaceutical-grade oltipraz; the cost for analytical-grade oltipraz is $59 per 5-mg sample and $236 per 25-mg sample (111). Though oltipraz can reduce aflatoxin bioavailability via several mechanisms, its cost makes it an economically impractical intervention. Second- and third-generation dithiolethiones are less expensive and more potent than oltipraz (126), but further studies on their potential side effects are needed.

Sulforaphane administration did not result in significant reductions in aflatoxin-N^7-guanine in two human intervention groups in China (high dose and very low dose), but interindividual variation in bioavailability was high. An inverse association was found between urinary levels of dithiocarbamates (sulforaphane metabolites) and aflatoxin-DNA adducts (85). Thirty sulforaphane capsules cost $25 (0.21% sulforaphane in 250 mg) (amazon.com). If consuming broccoli sprouts or a tea from these sprouts, a dose of 385-gram sprouts containing over 400 µmol glucoraphanin (to be metabolized to sulforaphane) costs about $0.31.

2.2.8 Costs and efficacy of hepatitis B vaccination

Because of the massive production of hepatitis B vaccines through improved biotechnologies, second-generation HBV vaccines' costs are much cheaper than the costs of the first generation vaccines. The vaccine's efficacies against HBV infection and chronic HBsAg carriage were 84–95% and 94–95%, respectively (112, 113). It is estimated that 53% of global HCC cases are attributable to HBV (114); therefore, we assume in table 2-3 that the corresponding reduction of HCC risk due to HBV vaccination ranges from 45–50%. Currently a dose of HBV vaccine costs

less than US $1 (127). It is estimated that HBV vaccination costs $910 for every death averted and $23 for every disability-adjusted life year (DALY) averted (115).

Currently available vaccines for HBV are multivalent vaccines. One of them is a pentavalent vaccine, in which HBV vaccine is combined with vaccines for diphtheria, tetanus, pertussis, and Haemophilus influenzae b (Hib). The five-in-one vaccine is believed to provide economic advantages over multiple immunizations of each monovalent preparation (128). The cost of the pentavalent vaccine is expected to drop to $2.94 per dose in 2010, 50 cents less than its cost in 2009 (129). Moreover, one of the major benefits of pentavalent vaccine; particularly in less developed countries where there is often a scarcity of health care personnel, is that the pentavalent vaccine reduces the total amount of time healthcare personnel spend to immunize children (128).

2.3 HOW TO ANALYZE COST-EFFECTIVENESS OF INTERVENTIONS

The World Health Organization (WHO) Commission for Macroeconomics and Health (130) provides the following guideline for thresholds of cost-effectiveness, as outlined in Wu and Khlangwiset (2010):

(1) An intervention is considered very cost-effective, if the monetary amount spent on the intervention per DALY averted is less than the *per capita* gross domestic product (GDP) for the nation in which the intervention is applied. In other words, the total cost of the intervention should be less than the product of the GDP and total DALYs averted.

(2) An intervention is considered moderately cost-effective, if the monetary amount spent on the intervention per DALY averted is less than three times the *per capita* GDP.

(3) An intervention is not cost-effective if, per DALY averted, its cost is greater than three times the *per capita* GDP.

The disability-adjusted life year (DALY) is a measure of the burden of disease. It includes both potential years of life lost due to premature death and years of "healthy" life lost in states of less than full health, broadly termed *disability* (131). The total number of DALYs associated with a disease is the sum of the years of life lost due to mortality from the disease

(YLL) and the number of years lived with a disability multiplied by a weighting factor between 0 and 1, depending on the severity of the disability (YLD):

$$DALY = YLL + YLD$$

Equation 1 Disability adjusted life year (DALY)

Wu and Khlangwiset (2010) estimate the cost-effectiveness of two aflatoxin control methods – biocontrol and postharvest drying and storage methods – in sub-Saharan Africa. By assuming a decrease in aflatoxin-induced HCC that is proportional to decreases in aflatoxin levels in maize preharvest and postharvest (for biocontrol) and aflatoxin-albumin adducts (for the postharvest intervention package), cost-effectiveness ratios (effectiveness in saving lives from cancer divided by cost of intervention) of 5.10–24.8 for biocontrol and 0.21–2.08 for the postharvest intervention package were estimated. Interventions whose cost-effectiveness ratios are greater than 1 can be deemed "very cost-effective" by WHO standards. These calculated ratios are actually underestimates of cost-effectiveness, because there are benefits to reducing aflatoxin other than decreasing liver cancer risk; there are benefits of improved immunity and reduced risk of stunting in children.

The cost information is usually presented in varying formats depending on the intervention in question; so cost-effectiveness analyses must be flexible, and care must be taken, to ensure that appropriate units are compared. For example, Wu and Khlangwiset (2010) started with cost data on biocontrol and postharvest intervention packages in two different formats: Biocontrol cost was given as a monetary amount per hectare treated, while the postharvest intervention package cost was given as a monetary amount to store 500–1,250 kg of groundnuts. To convert this into usable cost-effectiveness information, it was necessary to convert the costs using other data (e.g., amount of maize produced per hectare, amount of maize consumed on average per individual per year, number of households in Republic of Guinea) to estimate how many individuals were affected by the intervention every year.

It is important to choose appropriate health endpoints (i.e., effects) by which to evaluate DALYs. Aflatoxin, as described in the Introduction, has multiple different adverse health effects. The relationship between aflatoxin and HCC is the most well-established (132). DALYs

have been estimated for HCC, so this makes HCC prevention a convenient endpoint by which to evaluate the cost-effectiveness of aflatoxin risk reduction strategies. Stunted growth in children also has DALYs estimated for its societal impacts. Acute aflatoxicosis is a relatively less common effect associated with aflatoxin exposure. Immunosuppression is an extremely important effects associated with aflatoxin; however, the exact relationship is not as well characterized.

Table 2-4 lists the average annual GDP *per capita* in select nations across the world (133). These figures shed light on how feasible some of the aforementioned public health interventions would be in different parts of the world. In nations like Zimbabwe, where the average annual GDP per capita is $280 (USD), it is impossible for most individuals to afford an intervention that would cost more than a few cents per day; as the average daily income is less than $1. Even if the one–time cost of an intervention could improve health for years, it cannot be assumed that most families have saved enough money to be able to afford such an intervention. There are many competing demands for scarce resources, and often availability of food is more important than quality of that food. Moreover, a major challenge for any intervention in food-insecure countries is that there is little price differential for quality; hence, producers may have no incentive to invest in quality enhancement. It is likely that governments would need to pay for aflatoxin reduction interventions, at least in the foreseeable future.

Table 2-4. Average annual gross domestic product (GDP) per head in select countries across the world, in USD 2008.

Nation	Annual GDP per capita, USD 2008
Australia	$48,253
Canada	$45,166
China	$3,292
Cote d'Ivoire	$1,137
Kenya	$788
Nigeria	$1,450
Thailand	$4,187
United Kingdom	$43,544
United States	$45,230
Zimbabwe	$314

Note: Source: The Economist, 2007 (133), http://unstats.un.org/unsd/demographic/products/socind/inc-eco.htm

Ironically, individuals in industrial nations such as the US, United Kingdom, Canada, and Australia rarely directly pay the price to reduce aflatoxin–induced illness, whether by agricultural or clinical means. These costs are usually borne by growers (agricultural interventions to reduce aflatoxin) or by health insurance institutions or the national government (HBV vaccination).

2.4 DISCUSSION

The main purpose of this review is to bring together the scientific knowledge base (efficacies) and economic factors (costs, stakeholders) concerning aflatoxin risk-reduction strategies that could be deployed worldwide, and to highlight the importance of economic feasibility. Policy makers can use this information to decide: (1) whether the benefits (market and health) outweigh the costs of implementing the strategies, and (2) if so, then which stakeholders would pay the

costs and which would benefit in the long run, to resolve potential mismatches in economic incentives (39).

This information can also be useful to researchers who are developing further aflatoxin control strategies, in that they can roughly position their interventions among various existing strategies in terms of economic feasibility. It can also be useful to decision makers who want to weigh the relative importance of two categories of cost: the cost of preventing aflatoxin-related risks (to both markets and human health), and the cost of *not* preventing aflatoxin-related risks.

In preharvest settings, conventional breeding of maize and groundnuts to resist aflatoxin has shown great promise in terms of achievable efficacies. While initial research and development funding is of course necessary, once the resistant varieties are developed and disseminated, significant reduction of aflatoxin contamination can be achieved at very low, if any, additional cost to farmers. Replacement of local maize cultivars with agriculturally-improved varieties has been well-accepted by African farmers in recent history. It is estimated that a large part of 40% of present African maize yield is the result of planting improved cultivars (134). Transgenic crops that demonstrate aflatoxin reduction, on the other hand, may encounter several problems regarding wide-scale adoption worldwide. One problem is cost. Though the actual cost per acre for transgenic seeds may not be high, farmers may be required to buy new seeds each season if the seeds were developed in the private sector. Such an expense for farmers who are used to saving seed from season to season might be considered unacceptable. Another problem is governmental regulations against commercialization and trade of transgenic organisms in many parts of the world. Hence, transgenic technologies in agriculture are at the moment best-suited to nations in which it is already customary to buy new seed each season, and where biosafety laws permit planting of transgenic seeds.

Biocontrol through atoxigenic strains of Aspergilli has shown significant promise in controlling aflatoxin in a variety of crops in both preharvest and postharvest settings. Depending on the product, costs vary widely; but low-cost options are available in LDCs that have naturally occurring atoxigenic strains in their native soils. Biocontrol can be extremely cost-effective in reducing aflatoxin-induced disease (135) because of the protection against aflatoxin contamination that lasts for at least 6 months postharvest. As with transgenic crops, there may be regulatory issues to overcome in different nations, associated with the application of fungal strains to agricultural fields.

Good agricultural practices–those that can reduce various stresses on c rop plants and hence reduce fungal infection–can reduce aflatoxin contamination. Irrigation systems combined with insecticides can achieve extremely high efficacy in aflatoxin reduction, but capital costs to install the systems can be very high, as can operation and maintenance costs. These systems may not be affordable yet in many poorer LDCs.

In postharvest settings, physical methods to reduce aflatoxin accumulation are generally both less expensive and less risky than chemical methods. Physical sorting can remove the most contaminated food immediately postharvest. The postharvest intervention package described in Turner et al. (2005), which includes sorting as well as wooden drying pallets, natural-fiber storage bags, and insecticides, was estimated to be extremely cost-effective in reducing aflatoxin-induced HCC (135), without significant health or environmental risks. Chemical methods of destroying aflatoxin such as ammoniation and ozonation have extremely high efficacy levels at relatively low costs. However, handling ammonia can be dangerous if done improperly, and the process can cause reduced palatability and produce a byproduct which, though much less risky than AFB_1, may pose some health risks. Ozonation, because it appears to reduce protein efficiency in animals, may carry nutritional risks.

Dietary interventions to reduce aflatoxin risks can be considered forms of secondary prevention, as they do not actually reduce the amount of aflatoxin in the food, but can reduce its bioavailability in the body. NovaSil clay and chlorophyllin can both be produced at extremely low cost, and have shown significant reduction in biomarkers of aflatoxin-induced damage. Because both NS and chlorophyllin must be consumed at the same time as contaminated food in order to adsorb or sequester the aflatoxin, one potentially feasible mechanism is to blend these agents into a food item that is frequently used in local diets (e.g., maize meal). Green tea polyphenols would be an extremely cost-effective way to potentially reduce aflatoxin-induced health risks in cultures where green tea is already common in the diet. Otherwise, transportation costs and issues concerning the introduction of a relatively foreign drink (or pill) into the diet may render it impractical.

Chemoprevention through oltipraz and sulforaphane has shown some promise in reducing aflatoxin-induced HCC. Oltipraz is relatively expensive, however; and may not be practical as a long-term solution due to potential side-effects. With regard to obtaining sulforaphane from natural foods, at least two constraints exist: 1) the foods should ideally be locally produced, and

2) there is much variation in the concentration of the active compound in food. Therefore, it might be more reasonable to consider dietary chemoprevention as an additional intervention to other agricultural or clinical methods to reduce aflatoxin risks.

Hepatitis B vaccination has been employed in some degrees in Africa, initially with support from non–profit organizations such as the Global Alliance for Vaccines and Immunization (GAVI) and the Vaccine Fund (136). However, if support is withdrawn, each country has to determine the feasibility and costs of continuing this program within its own budget. As much of the infrastructure and basic materials needed for vaccination have been established during the initial phase, and inexpensive and effective vaccines are available, HBV vaccine programs are overall a useful, cost-effective, and feasible strategy to reduce aflatoxin-induced HCC (and indeed, HCC in general). However, the vaccine has no effect in those already infected with HBV. Hence, other aflatoxin-reduction methods are desirable, particularly in nations where HBV prevalence is high and HBV vaccination is still scarce.

Overall, efficacy tends to be higher for agricultural interventions (preharvest and postharvest) and for HBV vaccination than for dietary interventions, to reduce aflatoxin-related health risks. However, there are times in which only dietary interventions would be helpful, such as in the case of an emergency. For example, if an acute aflatoxicosis outbreak is occurring, it is too late to adopt agricultural interventions or to administer HBV vaccines to reduce aflatoxin's health effects – at least, to counteract the current crisis. Adsorbent compounds in the diet would make the most sense in such an emergency, if it is suspected that available food sources still contain dangerously high aflatoxin levels, and if the food cannot be simply discarded (e.g., for reasons of scarcity).

A limitation with the cost estimates of several of these interventions is that many of the costs reflect estimates from pilot studies (field or clinical trials) or anecdotal data. Actual costs done on a large scale for some interventions cannot be estimated, because some of the interventions have never been implemented on a large scale. Because of economies of scale, this is more likely to result in cost overestimates in this study, rather than underestimates. This highlights the need for further research to more accurately establish costs and efficacy of aflatoxin-risk reduction interventions worldwide.

A critical component to implementing any or all of these methods is community education (70). Not only should educational efforts include *how* to use the intervention properly

to achieve maximum benefit regarding aflatoxin risk reduction, it should also include *why* the interventions are important from health and market perspectives, so that users have incentive to continue with the interventions.

In summary, to reduce aflatoxin related problems in less developed countries, multiple types of interventions are potentially cost-effective; as they focus on di fferent targets, offer different outcomes, and achieve those outcomes under different time constraints. Understanding the costs, efficacy, and affected stakeholders of different aflatoxin control interventions can help decision makers–be they government policymakers or farmers or consumers–to optimally allocate resources, with the ultimate aim of improving public health.

3.0 EFFICACY OF INTERVENTIONS IN REDUCING AFLATOXIN-INDUCED LIVER CANCER: A RISK ASSESSMENT

The data presented in this chapter is prepared to submit for publication

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Pornsri Khlangwiset, Felicia Wu

Department of Environmental and Occupational Health, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA USA

3.1 ABSTRACT

Background: Chronic infection with hepatitis B virus (HBV) and high aflatoxin exposure in food synergistically predispose individuals to liver cancer (hepatocellular carcinoma, HCC) risk, especially in less developed countries where both risk factors are common. We wished to assess the relative efficacy of the HBV vaccine and two interventions intended specifically to control aflatoxin exposure in reducing aflatoxin-induced HCC.

Methods: We calculated annual and lifetime HCC burden caused by aflatoxin and chronic HBV infection by quantitative risk assessment of aflatoxin-induced liver cancer and age-standardized liver cancer rates in the 2010 Nigerian birth cohort. We used epidemiological and agricultural data to estimate the efficacy of the HBV vaccine compared with two aflatoxin-specific interventions, biocontrol and NovaSil clay, on reducing the burden of HCC associated with aflatoxin in Nigeria.

Results: 66-221 cases and 475-500 cases per 100,000 of the 2010 birth cohort are estimated to be attributable to aflatoxin and HBV, respectively. If 100% of the 2010 Nigerian birth cohort were vaccinated against HBV, the lifetime prevalence of aflatoxin-induced liver cancer in this population would be reduced by 77%. Biocontrol and NovaSil, if adopted continuously in the entire Nigerian population, would reduce HCC incidence by 5–19% and 3–11%, respectively. To prevent one aflatoxin-induced liver cancer case, about 587-1,970 infants must be vaccinated, 659–2,704 individuals must regularly consume biocontrol-treated maize, and 1,133–3,803 individuals must regularly consume NovaSil. **Keywords**: hepatitis B virus (HBV), aflatoxin, HBV vaccine, risk reduction, Nigeria, liver cancer

3.2 BACKGROUND

Liver cancer is the fifth most common cancer and the third most deadly cancer worldwide (137). Hepatocellular carcinoma (HCC) is the most common type of liver cancer. The number of new HCC cases is estimated at over 500,000 annually (138). In general, once diagnosed, patients will die in under a year without any treatment (139).

Globally, the geographic distribution of liver cancer is not uniform. The age-adjusted death rates of liver cancer are highest in sub-Saharan Africa (8.9–14.0 per 100,000) and East and Southeast Asia (14.9–24.0 per 100,000) (137). Three key risk factors for HCC in high-risk regions are chronic infection with hepatitis B virus (HBV) and hepatitis C virus (HCV) and foodborne aflatoxins (112, 140, 141). Globally, about 52% and 25% of HCCs are attributable to HBV and HCV, respectively (138). It has been estimated that aflatoxins may be responsible for 5%–28% of total liver cancer worldwide (142).

The role of HBV in causing HCC is supported by strong evidence from animal studies and epidemiological data, showing similar distributions of HCC and chronic HBV infection; higher detection rates of HBV surface antigen (HBsAg), a biomarker of chronic HBV infection, in HCC patients, compared with that in non-HCC populations; and evidence of chronic HBV infection prior to the development of HCC (143). However, the mechanism by which HBV induces liver cancer is still unclear and may involve both direct and indirect effects (144).

Aflatoxins, produced primarily by the fungi *Aspergillus flavus* and *A. parasiticus*, are found in multiple food commodities, most importantly maize and peanuts. One of the most potent naturally occurring human hepatocarcinogens, aflatoxin's highly reactive AFB_1 metabolite, AFB_1-8,9-exo-epoxide, intercalates in DNA and forms adducts with guanine, leading to base transversions in the p53 tumor suppressor gene.

Aflatoxin and chronic HBV infection synergistically increase the risk of HCC development (74, 145, 146). In less developed countries, the coexistence of foodborne aflatoxin and HBV makes liver cancer risk much higher than is experienced in industrial nations, where aflatoxin exposure and HBV prevalence are relatively lower. The mechanism of this synergy has not yet been elucidated. Researchers have discussed plausible mechanisms in several articles (147, 148). One concerns inflammatory effects of chronic hepatitis that may inhibit DNA repair mechanisms against aflatoxin-induced DNA damage. Another concerns the ability of HBV to induce enzymes in P450 systems involving the activation pathways of aflatoxin. Additionally, metabolic products generated by hepatitis viruses, such as reactive oxygen species or reactive nitrogen species, may favor aflatoxin-induced p53 mutations at codon 249^{ser} (147, 149).

Multiple interventions to reduce aflatoxin risk have been developed. The costs and efficacy of various types of aflatoxin control interventions have been reviewed (150). One of these interventions is biocontrol, in which atoxigenic *Aspergillus* strains are strategically applied

to crops to outcompete toxigenic strains, so that the crops may be infected with fungus, but there is little or no toxin. These biocontrol methods have been tested in maize, groundnut, and cotton fields in various parts of the world. Biocontrol can decrease aflatoxin levels in harvested crops by 70%–90% compared with untreated crops (58, 62, 96, 118, 151) . Importantly, atoxigenic *A. flavus* strains have been found in sub-Saharan Africa, which show promise for controlling aflatoxin in African maize (59, 62).

Another intervention that reduces aflatoxin risk is NovaSil: a dioctahedral smectite clay that, as a dietary additive, can reduce bioavailability of aflatoxins in humans and animals (70). This anti-caking agent adsorbs aflatoxin in the gastrointestinal tract between interlayer spaces and surfaces of clay molecules. In a human study in Ghana, NovaSil, at a 3-gram daily dose for 3 months, significantly reduced two biomarkers of aflatoxin exposure, aflatoxin M_1 and aflatoxin-albumin adduct, compared with the control (104).

Because the HBV vaccine reduces the risk of chronic HBV, it can reduce not just cancers caused by HBV alone, but also cancers caused by the synergism of aflatoxin and chronic HBV. Therefore, the HBV vaccine has been listed as an aflatoxin risk-reduction strategy (6, 150). Once infected by HBV, children are more likely to become chronic carriers than adults (152, 153); consequently, the infection could lead to premature deaths from cirrhosis and liver cancer. HBV vaccination in infants, therefore, is especially important for long-term protection against chronic HBV infection (154). Indeed, the HBV vaccine is believed to be one of the best preventive methods to control the spread of liver cancer. The HBV vaccine has been recommended for populations in areas with high liver cancer incidence (139, 149, 155).

Nigeria, the most populous country in sub-Saharan Africa, has a long history of aflatoxin contamination in food crops and chronic HBV infection (69, 156-159). The age standardized death rates of liver cancer in Nigeria ranges from 10.1 to 15.2 per 100,000 (160, 161). The purpose of our study is to quantitatively estimate burdens of aflatoxin-induced liver cancer and HBV-induced liver cancer in Nigeria; and to estimate the impacts of the HBV vaccine in comparison with other two aflatoxin control interventions, biocontrol and NovaSil clay, in reducing liver cancer burdens through three risk reduction estimates: absolute risk reduction (ARR), relative risk reduction (RRR), and number needed to treat (NNT).

3.3 METHODS

3.3.1 Liver cancer risk in Nigeria

3.3.1.1 HBV-induced liver cancer risk

We estimated the number of liver cancer cases in Nigeria based on the Nigerian age standardized death rate obtained from a World Health Organization (WHO) database (160) and the International Agency for Research on Cancer (IARC) GLOBOCAN 2008 database (161). As these rates are not HBV-specific death rates, we adopted the attributable fraction approach to determine the number of liver cancer cases attributable to HBV. The attributable fraction is the proportion of diseases that may be eliminated by getting rid of an exposure or a particular risk factor (114). It can be presented in a simple equation as follows:

$$AF = Pr\ (E, C\ )\ X\ ((risk\ ratio - 1)/risk\ ratio)$$

Equation 2. Attributable fraction *(114)*

Where AF = Attributable Fraction and Pr (E,C) = Prevalence of exposure among cases

The risk ratio for HBV infection resulting in liver cancer is often reported to be much greater than 10 (162-164). As the result, the attributable fraction is close to the prevalence of HBV infection among liver cancer cases (114); which, in Nigeria, ranges from 59% to 62% (165-168). These numbers are almost the same as the calculated attributable fraction (58.8%) of HBV on liver cancer in less developed countries reported by Parkin in 2006 (169), and 63%, the number of HBsAg+ (surface antigen biomarker of chronic HBV infection) in Gambian HCC patients (170).

3.3.1.2 Aflatoxin-induced liver cancer risk

We collected Nigerian data on aflatoxin levels in maize (69, 156, 157) and groundnuts (158, 159, 171), maize and groundnuts consumption rates per head (172), and prevalence of hepatitis B

chronic infection in Nigeria (173-189). Five year (2003–2007) maize and groundnut consumption rates per capita per day, obtained from the FAOSTAT database, were used to project consumption rates in 2010. The geometric mean levels of aflatoxin in maize and groundnuts based on available published literature were calculated. We divided detection limits by two for the samples in which aflatoxin was not detected to derive the lower range of aflatoxin levels. To obtain the upper range of aflatoxin levels, only positive samples were included.

Next, we estimated burdens of liver cancer induced by aflatoxin in HBV+ and HBV– populations in Nigeria. These numbers were calculated by cancer risk assessment methodologies, based on the aflatoxin cancer potency factors proposed by The Joint Expert Committee on Food Additives (JECFA) of the World Health Organization and Food and Agriculture Organization (145), and multiplied by the aflatoxin exposure data found above, assuming average bodyweight of 60 k g. JECFA proposed two carcinogenic potencies of aflatoxin: 0.30 cases per 100,000 individuals per year, per nanogram of AFB_1 intake per kilogram body weight per day, in the presence of HBV infections; and 0.01 corresponding cases per 100,000 without HBV infections (145). The difference between the number of HBV-induced liver cancer cases and the number of liver cancer cases caused by the synergistic effect between aflatoxin and HBV represents the number liver cancer cases caused by the interaction between chronic HBV infection and non-aflatoxin factors. Similarly, the difference between the numbers of non-HBV-induced liver cancer cases and aflatoxin-induced liver cancer cases in the HBV-negative population represents the number of liver cancer cases induced by non-aflatoxin and non-HBV factors. These other risk factors for liver cancer may include alcohol consumption, tobacco smoking, other dietary and environmental toxins, HCV infection, liver fluke, and metabolic syndrome (190).

3.3.1.3 Proportions of liver cancer induced by aflatoxin and HBV in relative to total liver cancer and the estimated numbers of liver cancer in the 2010 birth cohort in Nigeria

The proportions of liver cancer of these following groups, (I) aflatoxin/HBV+, (II) aflatoxin/HBV–, (III) non-aflatoxin factors/HBV+, and (IV) non-aflatoxin factors/HBV–, were calculated in relative terms to total liver cancer cases in Nigeria.

The baseline lifetime liver cancer risk was generated based on the mortality rates by age groups obtained from WHO (191) for the 2010 birth cohort. This baseline risk was multiplied by

the proportions of liver cancer of the 4 groups described above. We obtained the number of infants born in 2010 by multiplying the Nigerian population in 2010 by the Nigerian birth rate. From this, we subtracted the number of neonatal deaths, obtained by multiplying the number of infants by the Nigerian neonatal death rate, to estimate population size of the 2010 birth cohort.

We then estimated the number of infants perinatally infected with HBV and determined the number of infant deaths from HBV infection. The HBV infection mortality rate in newborns obtained from Goldstein et al. 2005 (192) was applied to the number of infants born with HBsAg+ to estimate the number of infant deaths from fulminant hepatitis.

We applied the 2008 Nigerian life table (193) to the 2010 birth cohort, from which the number of infant deaths from fulminant hepatitis was subtracted, to generate the expected numbers of deaths by HCC of the 2010 birth cohort at different ages: 0–14 years, 15–59 years, and >60 years, based on liver cancer death rates by age groups presented in the WHO Global Burden of Disease 2004 data (191). The numbers of liver cancer cases in different age groups were combined to obtain lifetime liver cancer cases in the 2010 birth cohort. The proportions of liver cancer induced by (I) aflatoxin/HBV+, (II) aflatoxin, (III) non-aflatoxin/HBV+, and (IV) non-aflatoxin/non HBV calculated earlier were applied to generate the numbers of liver cancer cases in different groups in the 2010 birth cohort, which were used as baseline risk

3.3.2 Efficacy of HBV vaccine and aflatoxin control methods in reducing liver cancer risk

The efficacies of biocontrol and NovaSil in reducing aflatoxin or aflatoxin-associated biomarkers were obtained from published literatures or personal communication, and applied to the baseline lifetime liver cancer risk induced by aflatoxin calculated earlier. It is widely accepted that the HBV vaccine is approximately 95% effective in reducing the prevalence of chronic hepatitis B carriage (HBsAg+) (115, 154). Therefore, 95% vaccine efficacy in reducing chronic HBV was used to determine number of liver cancer cases prevented by the vaccine in each age group. We applied 80% efficacy of biocontrol in reducing aflatoxin in maize in relative to total dietary aflatoxin exposure and 40% efficacy of NovaSil in reducing aflatoxin bioavailability to the numbers of liver cancer cases attributed to aflatoxin in the 2010 birth cohort to determine numbers of cases which would be prevented by the interventions.

Three risk reduction estimates, absolute risk reduction (ARR), relative risk reduction (RRR), and number needed to treat (NNT) of the HBV vaccine are calculated to determine the efficacy of the vaccine:

$$ARR = case\ prevalence\ (intervention-) - case\ prevalence\ (intervention+)$$

Equation 3. Absolute risk reduction (194)

$$RRR = ARR/case\ prevalence\ (baseline)$$

Equation 4. Relative risk reduction (194)

$$NNT = 1/ARR$$

Equation 5. Nuber needed to treat (194)

3.4 RESULTS

3.4.1 Liver cancer risk in Nigeria

3.4.1.1 HBV-induced liver cancer risk

Age standardized mortality rates of liver cancer in Nigeria, 10.1–15.2 per 100,000 (161, 191), were multiplied by 152 million, the number of Nigerian population in 2010 (195), to obtain the number of total liver cancer cases in 2010: 15,352–23,104 cases. Of these numbers, 9,058–14,324 cases are estimated to be HBV-related and 6,294–8,780 cases are non-HBV-related.

3.4.1.2 Aflatoxin-induced liver cancer risk

Table 3-1lists the studies surveying aflatoxin levels in maize and groundnuts or products made from these two commodities in Nigeria. In many cases, the maximum detected levels were higher than 20 ng/g, the maximum tolerable limit in Nigerian food, even when testing in preharvest grains. One recent study detected aflatoxin at levels up to 480 ng/g in maize tested within 23 days after being harvested (69). In dry-roasted groundnuts sold in markets in Nigeria, aflatoxin levels as high as 165 ng/g were detected. Percentages of aflatoxin positive maize samples ranged from 18.4% to 85%, which were relatively lower than those of groundnuts (64.2%–100%). Large variations of aflatoxin levels in food samples were detected in all but one study. The mean levels of aflatoxin in Nigerian maize and groundnuts ranged from 13.3 to 36.2 ng/g and 64.8 to 67.5 ng/g, respectively.

Table 3-1. Aflatoxin in maize and groundnuts in Nigeria

Commodity	Range (ng/g)[a]	Median (ng/g)[b]	% positive sample	Detection limit	Note	First author, Year
Maize	5–360	2.5	45%	5	Market	Adebajo 1994 (157)
Corn cake	5–345	25	85%	5	Market	Adebajo 1994 (157)
Corn roll	5–80	10	60%	5	Market	Adebajo 1994 (157)
Maize	3–130	<2	18.40%	5	Pre-harvest	Bankole 2004 (156)
Maize	1.1–480	4.2	>50%	1	Within 23 days after harvested, grains stored in paper bags	Bandyopadhyay 2007 (69)
Groundnuts cake	19–455	97.5	100%	NA	Market goods	Akano 1990 (159)
Groundnuts	5–165	NA	64.20%	5	Dry-roasted	Bankole 2004 (158)
Groundnuts	74.03–82.12	NA	100%	NA	Market goods	Odoemelam 2009 (171)

Note: [a]only aflatoxin levels of positive samples were included, [b]aflatoxin levels of all samples were counted

Maize and groundnut consumption rates in 2010 were about 73.6 g and 6.4 g per capita-day, respectively (172). Hence, aflatoxin exposure from consuming maize and groundnuts ranged from 16.37–44.36 ng/kgBW-day and 6.91–7.22 ng/kgBW-day, respectively. The estimated mean aflatoxin exposure from consuming both maize and groundnuts is 23.28–51.58 ng/kgBW-day.

While there are several studies to determine HBV prevalence rates in Nigeria, most of the studies focus on specific regions or specific groups of populations. In any case, a number of studies confirm high prevalence rates of the HBV infection in various populations in Nigeria (173-189); for example, HBV prevalence ranges from 13%–22% in blood donor groups (173-176), 2%–12% in pregnant women (176, 178-181), 18.7% in female sex workers (183), 23% in male prisoners (184), and 26%–52% in HIV positive individuals (173, 186-189). In this study, the Nigerian HBV prevalence is assumed to be 15%.

Aflatoxin exposure in Nigeria would result in 1.05–2.32 liver cancer cases per 100,000 HBV carriers, and 0.20–0.44 per 100,000 HBV-negative individuals annually. In the 2010 population of 152 million, 1,596–3,526 liver cancer cases per year in Nigeria are estimated to be due to the interaction between chronic HBV infection and dietary aflatoxin exposure, and 299–668 liver cancer cases per year would be due to dietary aflatoxin exposure alone.

3.4.1.3 Proportions of liver cancer induced by aflatoxin and HBV in relative to total liver cancer and the estimated numbers of liver cancer in the 2010 birth cohort in Nigeria

The estimated numbers of liver cancer cases distributed in these following groups: (I) aflatoxin-induced liver cancer in individuals with chronic HBV infections, (II) aflatoxin-induced liver cancer in individuals without HBV infections, (III) non-aflatoxin-induced liver cancer in HBV carrier population, and (IV) non-aflatoxin-induced liver cancer in HBV-negative population are presented in table 3-2.

Table 3-2. Age standardized attributable risk of aflatoxin and hepatitis B infection on liver cancer in Nigeria

Liver cancer		HBV +		HBV -	Total
AF-related	I	1,596–3,526 *(7%–23%)*	II	299–668 *(1%–4%)*	1,895–4,194 *(8%–27%)*
Non-AF-related	III	5,532–12,728 *(36–55%)*	IV	5,626–8,481 *(35%–39%)*	11,158–21,209 *(73%–92%)*
Overall		9,058–14,324 *(59–62%)*		6,294–8,780 *(38–41%)*	15,352–23,104 *(100%)*

Note: Population =152 million

Aflatoxin exposure accounts for about 8% to 27% of total liver cancer cases in Nigeria. Aflatoxin exposure in the presence and absence of chronic HBV infection account for 7%−23% and 1%−4% total liver cancer cases in Nigeria, respectively.

Given the population in 2010 in Nigeria of 152 million (196), Nigerian birth rate of 36.65 per 1,000 (197), and neonatal death rate of 47 per 1,000 (198); the population of the 2010 Nigerian birth cohort is approximately 5.3 million. Because 13–30%, of HBsAg-positive mothers contained HBeAg (176, 199), of the 5.3 million, about 20,800–48,000 infants of 5.3 million infants born in 2010 are expected to born with HBV seropositive caused by perinatal contamination. In any case, the likelihood of children to develop fulminant hepatitis is less than 10 in a million (192). None of the 2010 Nigerian neonates would be expected to die from perinatal acute HBV infection (data not shown).

Table 3-3 shows the estimated lifetime risk to develop liver cancer in the 2010 birth chort. About 43,000 (806 per 100,000) liver cancer cases would be developed during the lifetime of the 2010 birth cohort. The estimated numbers of lifetime HBV-related liver cancer cases in the 2010 birth cohort would range from 25,372 to 26,662. The lifetime numbers of non-HBV related liver cancer cases would range from 19,081 to 20,587 cases.

Table 3-3. Estimated lifetime risk to develop liver cancer and health impacts of the HBV vaccination in the 2010 Nigerian birth cohort

	HBV+			HBV-			Total cases reduction
	Baseline	Hepatitis B vaccine	# cases prevented	Baseline	Hepatitis B vaccine	# cases increase	
AF-induced	2,951–9,902	148–495	2,804–9,407	557–1,870	651–2,184	(94–314)	2,710–9,093
Other factors (X)–induced	15,470–23,711	774–1,185	14,696–22,525	15,761–15,784	18,403–18,430	(2,642–2,646)	12,054–19,879
Overall	25,372–26,662	1,269–1,333	24,103–25,329	16,341–17,631	19,081–20,587	(2,740–2,956)	**21,147–22,589**

Note: numbers in brackets indicate that numbers in groups after vaccination program higher than baseline (negative values). 2010 birth cohort population =5,336.8 thousand

3.4.2 Efficacy of HBV vaccine and aflatoxin control methods in reducing liver cancer risk

In the 2010 birth cohort, the vaccine is expected to prevent liver cancer in HBV positive group by 24,103–25,329 cases (see table 3-3). Of these numbers, the vaccine would prevent aflatoxin-induced liver cancer in the HBV population by 2,804–9,407 cases. The increased number in HBV-negative population, as a result of the vaccine, would lead to the increased number of liver cancer in non-HBV carriers by 2,704–2,956 cases. Overall the vaccine would prevent 2,710–9,093 cases from aflatoxin-related liver cancer, 24,103–25,329 cases from HBV-induced liver cancer by, and 21,147–22,589 total liver cancer cases.

Table 3-4. Expected health impacts of select aflatoxin control interventions on liver cancer in the 2010 Nigerian birth cohort

	No. of cases without interventions	Biocontrol		NovaSil	
		No. of cases	No. of cases prevented	No. of cases	No. of cases prevented
HBV+	2,951–9,902	1,282–3,102	1,669–6,800	1,771–5,941	1,180–3,961
HBV–	557–1,870	242–586	315–1,284	334–1,122	223–748
Total	3,508–11,772	1,524–3,688	**1,984–8,084**	2,105–7,063	**1,403–4,709**

Table 3-4 lists the expected number of liver cancer cases prevented by the two aflatoxin control strategies biocontrol and NovaSil clay. Unlike the HBV vaccine, both biocontrol and NovaSil can reduce liver cancer risk in either HBV-positive or HBV-negative populations. Biocontrol and NovaSil prevent about 2,000–8,000 and 1,400–4,700 liver cancer incidents, respectively, over the lifetime courses of the 2010 birth cohort.

Overall, the HBV vaccine reduces the greatest numbers of total liver cancer compared with biocontrol and NovaSil. Of 43,000 total liver cancer cases, the HBV vaccine, biocontrol, and NovaSil reduce liver cancer by 49%–53% (21,147–22,589 cases), 5%–19% (1,984–8,084 cases), and 3%–10% (1,403–4,709 cases), respectively.

Table 3-5. Risk reduction estimates of selected control interventions in liver cancer by etiology

Items	Interventions	Aflatoxin-related liver cancer	HBV-induced liver cancer	Overall liver cancer
Baseline risk (per 100,000)		65.73–220.58	475.42–499.59	805.78
ARR (per 100,000)	Vaccine	50.76–170.39	450.44–475.81	395.05–424.47
	Biocontrol	37.17–151.47	31.27–127.41	36.98–151.82
	NovaSil	26.29–88.23	22.12–74.22	26.30–88.23
RRR	Vaccine	0.77	0.95	0.49–0.53
	Biocontrol	0.57–0.69	0.06–0.27	0.05–0.19
	NovaSil	0.40	0.04–0.16	0.03–0.11
NNT	Vaccine	587–1,970	210–222	236–253
	Biocontrol	660–2,690	785–3,198	659–2,704
	NovaSil	1,133–3,803	1,347–4,521	1,133–3,803

To summarize: per 100,000 populations, the HBV vaccine, if 100% adopted is estimated to prevent 51–170 (77%: 51/66, 170/221) and 450–476 (95%: 450/475, 476/500) cases from developing liver cancer induced by aflatoxin and HBV, respectively. About 395.05–424.47 (49–53%) cases in 100,000 would be prevented from developing liver cancer, regardless of cause. In order to prevent one liver cancer case, regardless of cause, 236 to 253 infants need to be vaccinated. About 587 to 1,970 and 210 to 222 infants need to be vaccinated to prevent one liver cancer case caused by aflatoxin and HBV, respectively.

About 6%–27% and 4%–16% of HBV-induced liver cancer incidents are expected to be reduced by biocontrol and NovaSil, respectively. NovaSil would reduce liver cancer by 3%–11% of total liver cancer. Approximately 5%–19% of total liver cancer would be avoided if biocontrol was adopted. The HBV vaccine reduces total liver cancer by about 50%. The ARRs of biocontrol and NovaSil indicate that they reduce fewer liver cancer cases, compared with the HBV vaccine,

as a result these two aflatoxin control interventions requires greater numbers of individuals to adopt these interventions than the HBV vaccine.

3.5 DISCUSSION

In general, the incidence of liver cancer increases after 20 years of age, then reaches a peak and stabilizes around the age of 50 (200). At present, the life span of a Nigerian is estimated to be 47 years old (201). If overall Nigerian public health were improved, leading to longer life expectancy, the lifetime risk of liver cancer could become higher than the numbers presented in this study.

Though liver cancer is a multi-factorial disease, we show that a significant portion of liver cancer cases in Nigeria is associated with HBV infection, aflatoxin, or both. HBV vaccination in infants would greatly reduce the numbers of liver cancer cases in Nigeria either induced by HBV alone, or a combination of aflatoxin and HBV. Though some regions have high vaccine uptake rates, overall national vaccination rates are still low (202, 203), at about 41% (198). Among Global Alliance for Vaccines and Immunisation (GAVI) eligible countries, Nigeria was ranked second after India in nations that have the highest proportion of children unvaccinated against HBV (204). One study estimated that only 1% of children whose mothers were market traders in Ibadan were immunized against HBV (205). Our analyses reveal that the HBV vaccine reduces a greater number of liver cancer cases than two effective aflatoxin control strategies. Aside from liver cancer, one of the advantages of the HBV vaccine is the reduction in premature deaths caused by HBV-related conditions and diseases, such as acute HBV infection and HBV-induced cirrhosis.

One of the limitations of the study is the estimation of aflatoxin exposure. Hall and Wild (1994) described this difficulty in 1994, and discussed how biomarkers could resolve some problems related to obtaining accurate aflatoxin exposure data (206). In the intervening time, several serum- and urine-based biomarkers of aflatoxin exposure, internal dose, and biologically effective dose have been validated in experimental models and epidemiological studies (74). Use of these biomarkers has greatly assisted epidemiological studies. However, these have not

been collected in the broad Nigerian population, so aflatoxin exposure was estimated from food samples and estimates of food intake. Limits of detection of aflatoxin analysis methods may affect the number of positive samples found in assays. Bandyopadhyay et al. (2007) identified aflatoxin in more than 50% of maize samples using the method that can detect aflatoxin levels as low as 1 ng/g, while only 18.4% and 45% of pre-harvested maize and maize sold in markets were found to be aflatoxin-contaminated in studies using less sensitive techniques.

The results of this study should not be interpreted as recommending greater HBV vaccine uptake *in lieu* of aflatoxin control interventions. Aflatoxin causes adverse health and economic impacts beyond liver cancer. Although the HBV vaccine can reduce hepatotoxicity caused by the HBV infections, or by the synergistic effects between aflatoxin and the HBV infection, it cannot reduce aflatoxin in food or feed staples. Therefore, the vaccine does not prevent other toxicities of aflatoxins, such as aflatoxicosis, growth impairment, or immunosuppression. Moreover, aflatoxin may compromise markets and trade due to unacceptable levels in food (38). One important characteristic of aflatoxin-specific interventions is that either HBsAg- or HBsAg+ individuals do benefit from them, unlike the HBV vaccine, which only provides benefits to those who are or were not already HBV-infected. An individual who is already chronically infected with HBV does not benefit from the HBV vaccine. Therefore, both the vaccine and specific aflatoxin control strategies are needed to improve health outcomes in Nigeria.

4.0 AFLATOXIN CONTROL INTERVENTIONS: COST-EFFECTIVENESS ANALYSIS

4.1 ABSTRACT

The costs and health-based efficacy of four aflatoxin control interventions: biocontrol, the postharvest intervention package, NovaSil clay, and hepatitis B vaccine in Nigeria are evaluated. The benefits of preventing liver cancer are presented as the numbers of disability life years (DALYs) averted. A 3% discount rate was applied to the benefits of preventing liver cancer to convert benefits of preventing liver cancer in the future to current values. The cost effectiveness ratios (CERs)–the costs to reduce one disability life year–range from $1,073 to $1,146 for the HBV vaccine to $26,467 to $69,110 for biocontrol. Regarding WHO criteria, the HBV vaccine is a worthy intervention to reduce liver cancer in Nigeria (CER<3GDP per capita). Biocontrol could be a worthy or non-worthy intervention depending on aflatoxin levels in staple crops. Either reductions in cost or improvements in efficacy are needed for the postharvest intervention package and NovaSil clay to be a worthy intervention to reduce liver cancer in Nigeria.

4.2 INTRODUCTION

In the previous chapter, we have shown that aflatoxin and the chronic hepatitis B virus (HBV) infection are responsible for 8–27% and 59–62% of liver cancer cases, respectively in Nigeria. We also determined the health impacts of the HBV vaccine and the other two aflatoxin control interventions on lifetime liver cancer in the 2010 birth cohort. Though the efficacy of each intervention differs from 3–11% in NovaSil to 49–53% in the HBV vaccine, it can still be

difficult for an individual to determine whether any of these interventions is worthy to be adopted.

Understanding the costs and efficacy of different aflatoxin control interventions can help decision makers–be they government policymakers or farmers or consumers–to optimally allocate resources, particularly in conditions of scarcity. At least three techniques can be used as a tool to compare benefits and costs of different types of public health interventions. These three techniques include cost-benefit analysis (CBA), cost-effectiveness analysis (CEA), and cost-utility analysis (CUA).

These three techniques measure outcomes differently. In CBA, both cost and benefits are often presented in monetary units; whereas in CUA, the benefits are often expressed in terms of quality of life or life year gained. In contrast, the outcomes in CEA are generally clinical effects (207).

Comparing interventions can be a problem if the clinical outcomes differ. Therefore, many studies utilize the concept of disability adjusted life year (DALY). DALYs for a disease are the sum of the present value years of life lost due to the premature deaths (YLL) and the years lost due to disability (YLD) for incidents of disease or injury (208). One DALY equals one healthy life year lost.

Because DALY is a time-based measure, DALYs are affected by time discount rate for the future outcome and age. Fewer DALYs are assigned if an incident happens to children or the elderly than middle aged persons. Moreover, men and women are assigned different numbers for DALYs even when they have the same disease (209).

The World Health Organization (WHO) recommended the gross domestic product (GDP) per capita as a readily available threshold value to classify an intervention into one of the following three cost-effectiveness categories: highly cost-effective, cost-effective, and not cost-effective (130).

We previously determined the cost-effectiveness of two aflatoxin control interventions; biocontrol in Nigeria and the postharvest intervention package in Guinea. If the benefits were projected to occur five years after the aflatoxin levels in staple crops were reduced by these two interventions, either biocontrol or the postharvest intervention package would be cost-effective (135). In this study, we extended the expected time that benefits occur to 10 years, included the HBV vaccine and NovaSil clay in the analysis and compared these four interventions if they

were adopted in the selected country, Nigeria. Our objective was to determine whether these aflatoxin control interventions were worthy to be implemented in Nigeria using the cost-effectiveness assessment method. Intervention characteristics, costs, and efficacy of various types of aflatoxin control interventions including biocontrol, the postharvest intervention package, NovaSil clay, and the HBV vaccine are intensively discussed in our previous work (150).

Biocontrol is the name given to atoxigenic Aspergilli deliberately applied to crops to outcompete the toxigenic Aspergilli, so that the final crops may be infected with fungus, but there is little or no toxin. These biocontrol methods have been tested in maize, groundnuts, and cottonseed worldwide. A number of non-toxin producing fungi have been identified and tested in various countries. Importantly, atoxigenic *A. flavus* strains have been found in sub-Saharan Africa, which show promise for controlling aflatoxin in African maize (59, 62). Afla-safe^{TM} is a biocontrol developed by the International Institute for Tropical Agriculture (IITA) based in Nigeria. Sorghum is used as the substrate of Afla-safe^{TM}. Currently, Afla-safe^{TM} offers about 80% response in reducing aflatoxin contamination in Nigerian maize field trials (Dr. Ranajit Bandyopadhyay, IITA plant pathologist, personal communication).

To improve drying and storage conditions of groundnuts in Guinea, Turner et al. 2005 incorporated several components into their postharvest intervention package. These components included educating the public on how to sort and dry nuts; using materials that enhance air ventilation, such as fibre mats to dry nuts, and fibre bags to store nuts; using a wooden pallet on which to store bags; and applying insecticide on storage floor to control pests in storage structures (67). Turner et al. (2005) tested this package in Guinea and found a reduction of 69% in the mean aflatoxin level in groundnuts (67).

NovaSil clay is an anti-caking agent in animal feed. It reduces bioavailability of aflatoxin by adsorption of aflatoxin in the gastrointestinal tract between interlayer spaces and surfaces of the clay molecules. Based on a study done in Ghana, NovaSil clay, at a three-gram daily dose, can reduce aflatoxin bioavailability by 40% (104).

The hepatitis B vaccine is believed to be one of the best preventive methods to control the spread of liver viruses, which together with aflatoxin, greatly increase the liver cancer risk above the risk of either risk factor alone. At present, the third generation recombinant hepatitis B vaccines are cheaper than prior generations. The monovalent hepatitis B vaccine costs $0.21 per

dose (210)–less than one dollar per three-dose course. However, in Africa where percentages of primary vaccination coverage are low, the Global Alliance for Vaccines and Immunisation (GAVI)–a global partnership between the public and private sectors, e.g. WHO, UNICEF, the World Bank Group, Bill & Melinda Gates Foundations, etc–provides supports for pentavalent vaccines over the monovalent vaccines.

The pentavalent vaccine requires three dose vaccinations, similar to the monovalent hepatitis B vaccine, but aside from HBV infections, the pentavalent vaccine provides protective effects to other four diseases: diphtheria, tetanus, pertussis, and hemophilus influenza type b (Hib). However, the cost of the pentavalent vaccine is much higher than monovalent vaccines, but in 2009, it was projected to be reduced to $2.94 per dose in 2010 (129).

4.3 METHOD

We determined efficacy of aflatoxin control interventions in reducing liver cancer of an implementation of a particular intervention if it were 100% adopted.

4.3.1 Cost-effectiveness analysis

We performed the CEA by introducing the approach of cost-effectiveness ratio (CER), which is the ratio between the costs of interventions and expected outcomes. The numbers of bad incidents prevented are multiplied by average burdens per case, obtained from the 2004 global burdens of diseases (208). Regarding the JECFA's aflatoxin carcinogenic potencies, the effects of aflatoxin were calculated as annual liver cancer cases per 100,000. We assumed that the effects would happen at the tenth-year after the interventions were introduced. But for the HBV vaccine, the protective effects are life-long. The numbers of prevented DALYs were discounted by 3% per year to account for future benefits from current investment.

4.3.1.1 Costs of interventions

The costs of interventions are the annual cost of each intervention if they are 100% adopted in Nigeria. The aflatoxin control interventions, including biocontrol, the postharvest intervention package, and NovaSil, must be repeated annually or infants must be given three dosages for the HBV vaccine.

4.3.1.2 Efficacy of interventions

Aflatoxin-induced liver cancer: The Nigerian aflatoxin exposures per kilogram bodyweight were calculated based on aflatoxin levels in maize and groundnuts, and the Nigerian consumption rates of these two major staples. Quantitative liver cancer risk assessments in Nigeria in the presence and absence of a particular intervention were performed regarding the number of Nigerian population in 2010 and carcinogenic potencies of aflatoxin proposed by JECFA. These were 0.3 per 100,000 per year for a nanogram of aflatoxin intake per kilogram body weight for an individual chronically infected by the HBV and 0.01 in a corresponding case for a HBV-free individual (132).

HBV-induced liver cancer (HBV vaccination): The 2010 cohort population structure was generated using the calculated number of survival infants born in 2010 and applied to the 2008 Nigerian life table (193). Then the mortality rates by age groups were applied to the 2010 cohort to generate estimated liver cancer throughout the 2010 cohort lifetime, which is the base line liver cancer risk in this population. Later, we calculated the expected number of liver cancer cases prevented based on 95% efficacy of the HBV vaccine to prevent chronic HBV infection and the attributable fraction of chronic HBV infection on Nigerian liver cancer which is about 59-62%.

4.3.1.3 Cost-effectiveness ratio (CER)

The CER– the ratio between the cost and the benefit of a particular intervention to determine the cost to prevent one healthy life year lost–of each intervention was compared with the Nigerian GDP per capita, which was about $2,360 in 2010 (211). Based on this ratio, each intervention

was assigned to one of three following categories proposed by WHO (130): highly cost-effective (CER< one GDP per capita), cost-effective (one GDP<CER<three GDP), and not cost-effective (CER> three GDP per capita).

4.4 RESULTS

4.4.1 Costs of interventions

4.4.1.1 Biocontrol

The cost of Afla-safe^{TM} –biocontrol developed by IITA and tested in Nigeria–is about $18 per hectare (2010 Personal communication Dr. Ranajit Bandyopadhyay, IITA plant pathologist). This cost includes material and distribution costs. The 2010 Nigerian maize planting area projected from the FAO 2004–2008 databases (212) is approximately 4.19 million hectares. Therefore, the total cost of biocontrol if this intervention were applied into all maize fields in Nigeria would be about $75.40 million.

4.4.1.2 The postharvest intervention package

The cost of the postharvest intervention package proposed by Turner et al. 2005 is $50 to store 500–1,250 kg of groundnuts (67). In 2010, this package would cost about $65.03. The purchasing power parity (PPP) approach was employed to convert the cost of the package in Guinea to the cost of this package in Nigeria. Purchasing power parity (PPP) is the exchange rate that equates the price of a basket of identical traded goods and services in two countries. PPP is an extension of the law of one price (LoOP) –one of the most basic laws in economics– which states that identical goods should be sold at the same price in two different markets if there is no transportation cost and no difference in tax rate between these two markets. The simplest way to think of the PPP is to compare the cost of "a standard good" that identical across countries. Hamburger index, which is the price of Mcburger around the world is one of the most typical samples of the PPP approach (213). PPPs are often very different from the current market

exchange rates. While market exchange rates reflect short-term relative value of different currencies, PPPs provide information of long-term value.

Table 4-1. 2005 Purchasing power parity (PPP) and market exchange rate of Nigeria and Guinea

Country	PPP (1 $US) (214) in local currency unit	Exchange rate (1 $US) (215)
Guinea	1,219.35 GNF	3,644.33 GNF
Nigeria	60.23 Naira	131.27 Naira

Note: (PPP= purchasing power parity) GNF = Guinean Franc,

$50 USD = 50 x 3,644.33 (GNF) = 182,216.50 GNF

Using PPP to normalize to standardize USD 182,216.50/ 1,219.35 GNF per 1 USD

= 149.44 USD

The PPP of $149.44 is similar to = 149.44 x 60.23

= 9,000.77 Nigerian naira

9,000.77 PPP of Nigerian naira converted to USD based on exchange rate

= 9,000.77/131.27

= 68.57 USD in 2005

The cost of the postharvest intervention in 2005 converted to 2010 currency value

= $ 89.19

The 2010 groundnuts production in Nigeria was about 4.32 million tons. This number was projected based on the FAO 2004–2008 databases (212). The estimated costs of the package per year to store this amount of groundnuts range from $103.68 million to $259.20 million; assuming the package lasts for three years.

4.4.1.3 NovaSil clay as a dietary preventive agent

Currently, the most effective dose of NovaSil clay to reduce aflatoxin bioavailability tested in humans is three grams per meal. The cost of NovaSil clay was $0.67 per kilogram in 2009 (2009 Personal communication: Dr.Timothy Phillips) which was about $0.71 per kilogram in 2010. With three meal daily basis and the number of Nigerian population in 2010 of 152 million, about 166.44 million kilograms or 166,440 tons of NovaSil clay were required to serve the Nigerian population in 2010 leading to a $117.54 million cost of the clay. Unlike other previous interventions, at present, there is no known source of NovaSil clay in Nigeria. The costs to export NovaSil clay from the USA, transport, and import to Nigeria are included in our analysis, assuming the clay is exported from USA to Lagos, Nigeria.

Table 4-2 lists the parameters used for calculating the cost of NovaSil clay. The loose bulk density of NovaSil clay is 40 lb per $ft.^3$ (216). The dimensions of standard 20-ft. container are 19' 4"x 7' 8"x 7' 9"(217). After a 10% deduction of capacity of standard containers for packaging and internal capacity, about 8,800 of 20-ft. containers are required. The costs of export from the US and import to Nigeria per 20-ft. container measured the fees levied on a 20-ft. container are $1,050 (218), and $1,440 (219)[1], respectively. The freight transport cost to transport 8,800 of 20-ft containers from the USA to Lagos Nigeria is $39.53 million (Online freight calculator for a full load container 2010 May 11). Johns et al., 2003 estimated that, in general, the cost of domestic transport would be about 1-3% of original product cost (220). In this study, the domestic transport cost is assumed to be 2% of original cost. Therefore, NovaSil would cost $181.33 million per year, assuming NovaSil clay is shipped in 20-ft. containers.

[1] All the fees associated with completing the procedures to export or import the goods are included. These include costs for documents, administrative fees for customs clearance and technical control, customs broker fees, terminal handling charges and inland transport. The cost measure does not include tariffs or trade taxes.

Table 4-2. Cost of NovaSil clay per year in Nigeria

Items	Results	References
Direct cost of intervention		
Cost of NovaSil clay($)	$0.71/kilogram (3 grams per meal dose) ($0.73 per person per year)	Dr. Timothy Phillips Personal communication, author calculation
2010 Nigerian population	152 million	(201)
Total cost per year ($)	117.54 million	Author calculation
Transport cost		
Loose bulk density of the clay (per $ft.^3$)	40 lbs.	(216)
Percent reduction of capacity due to packaging and internal capacity	10	Author assumption
Number of 20-ft. container required	8,800	Author calculation
Export cost from USA per a 20-ft. container ($)	1,050	(218)
Import cost to Nigeria per a 20-ft. container ($)	1,440	(219)
Freight transport cost from USA to Lagos Nigeria for 8,800 of 20-ft. containers ($)	39.53 million	Online freight quote for full load container 2010 May 11
Domestic margin ($)	2.35 million	Author calculation
Total shipping cost ($)	63.51 million	Author calculation
Total cost (clay+shipping) ($)	181.33 million	Author calculation

4.4.1.4 Hepatitis B vaccine

Table 4-3 listed all required parameters used for calculating the cost to immunize the 2010 birth cohort against the HBV infections. Given reserve stock of the vaccine to be zero, the cost of pentavalent vaccine was expected to be $2.94 per dose in 2010 (129). The average costs per children without vaccines range from $5.9 to $8.8 (221). These non vaccine cost, based on the data from eight countries, in which vaccine coverage rates were either low (< 50%) or high (>90%), were converted to 2010 values–$8.99 to $13.40.

In 2009, the estimated Nigerian birth rate was 36.65 per 100,000; whereas, the population of Nigeria in 2009 was 149.23 million (201). Therefore, the estimated number of children born in 2010 was about 5.47 million. The costs of the pentavalent vaccine and non vaccine were divided

by three to reflect the cost of the HBV vaccine. The overall costs in 2010 to HBV vaccinate all new born children in Nigeria ranged from $37.83 million to $ 45.88 million.

Table 4-3. Cost of HBV vaccination per year in Nigeria

Items	Results
Cost of intervention ($)	
Vaccine cost ($)	2.94 per dose (129)
Numbers of dose required	3
Wastage rate	25% (222) for liquid vaccines in a 10 or 20 dose vials
Total ($)	21.44 million
Non vaccine cost ($)	8.99–13.40 per dose (221)
2009 Nigerian population	149.23 million (201)
Birth rate (per 1,000)	36.65 (201)
Number of infants born in 2010	5.47 million
Total ($ millions)	16.38 to 24.44
Total cost of HBV vaccination program ($ millions)	37.82 to 45.88

4.4.2 Efficacy of Interventions

4.4.2.1 Aflatoxin control interventions

In our previous chapter, we collected the data of aflatoxin levels in Nigerian maize and groundnut goods from various sources (69, 156, 157, 159, 171) and showed that per kilogram bodyweight per day, a Nigerian is exposed to 16.37–44.34 and 6.91–7.22 nanogram of aflatoxin from consuming maize and groundnuts contaminated with aflatoxin. Together with the high prevalent of chronic HBV infections (173-189)–Assuming the HBV prevalence rate of 15%–without any interventions, aflatoxin exposure in Nigeria would induce liver cancer developments by 1.25–2.76 per 100,000 per year. Of these numbers 0.88–2.37 cases per 100,000 per year are the results of consuming maize contaminated with aflatoxin and 0.37–0.39 case per 100,000 are due to aflatoxin exposure in groundnuts.

Table 4-4 presents estimated numbers of liver cancer cases and disability life years lost which could be prevented if each of the three aflatoxin control interventions were adopted. A standard 3% discount rate was applied for ten years to obtain current DALYs values. On average, a Nigerian consumes maize in a larger amount compared with groundnuts (76.53 grams/day versus 6.46 grams/day) (172). Biocontrol, the postharvest intervention package, and NovaSil clay would reduce aflatoxin-related liver cancer cases by 1,065–2,886, 388–405, and 758–1,677, respectively. All of these interventions can provide thousands DALYs averted depending on levels of aflatoxin in food crops.

Table 4-4. Estimated numbers of preventable liver cancer cases and DALYs averted from reducing aflatoxin exposure by select aflatoxin control interventions

Items	**Biocontrol**	**The postharvest intervention package**	**NovaSil clay**
Target staple crops	Maize	Groundnuts	Not specific
Efficacy of intervention	80% (M)	69% (G)	40% (NS)
AF exposure per kgBW-day (ng) due to maize consumption (Intervention +)	3.27–8.87	16.37–44.34	9.82–26.60
AF exposure per kgBW-day (ng) due to groundnuts consumption (Intervention +)	6.91–7.22	2.14–2.24	4.15–4.33
Total aflatoxin exposure (Intervention+)	10.18–16.09	18.51–46.58	13.97–30.93
HCC cases predicted per 100,000 (intervention+)	0.54–0.86	0.99–2.49	0.75–1.65
HCC cases prevented per 100,000	0.70–1.90	0.26–0.27	0.50–1.10
Total HCC cases prevented	1,065–2,886	388–405	758–1,677
Total DALYs saved	13,842–37,514	5,047–5,261	9,849–21,799
Total DALYs saved (adjusted)[a]	10,300–27,914	3,755–3,915	7,328–16,220

Note: [a]DALYs were adjusted by discounting 3% for the effects assumed to begin at the 10th year. Total population of Nigeria in 2010 = 152 million (196).

4.4.2.2 Hepatitis B vaccine

Liver cancer in 2010 birth cohort

In the previous chapter, we estimated the numbers of liver cancer cases in the 2010 birth cohort in Nigeria and the reduced liver cancer burdens when the HBV vaccine was 100% adopted in this population. Given the Nigerian liver cancer death rates by age groups (191) and the attributable fractions of HBV infections, 59–62%, on liver cancer, the HBV infections would induce liver cancer by 0.10, 3.11–3.27, and 47.92–50.36 per 100,000, in populations aged between 0–14, 15–59, and older than 60 years old age, respectively. The DALYs given to a liver cancer case developing at the age 0–14, 15–59, and older than 60 years old are: 30, 20, and 7, respectively.

Table 4-5. Estimated lifetime health impacts of the HBV vaccination in 2010 Nigerian birth cohort

Age	Baseline		HBV vaccine		# reduced cases	DALYs	Adjusted DALYs
	HBV+	HBV-	HBV+	HBV-			
0–14	63–66	40–44	3	47–51	53–56	1,587–1,680	1,290–1,378
15–59	5,085–5,344	3,275–3,534	254–267	3,824–4,126	4,238–4,528	84,760–90,555	28,393–30,329
>60	20,224–21,252	13,025–14,054	1,011–1,063	15,209–16,410	16,856–18,006	110,214–117,730	10,358–11,064
Overall	25,372–26,662	16,340–17,632	1,269–1,333	19,079–20,588	21,147–22,591	196,561–209,965	40,041–42,771

Note: Population = 5.07 million

Table 4-5 shows the estimated lifetime health impacts of the HBV vaccination in the 2010 birth cohort. Overall the vaccine would reduce liver cancer in 21,147 to 22,591 cases. The estimated DALYs averted from liver cancer by adopting the HBV vaccine range from 196,561 to

209,965. With the 3% discounted rate, the vaccine would prevent about 40,041 t o 42,771 disability adjusted life years lost.

Table 4-6. Potential DALYs prevented and cost effectiveness ratios (CERs)

Items	**Biocontrol**		**The postharvest intervention package**		**NovaSil clay**		**HBV vaccination**	
	Lower bound	Upper bound	Lower bound	Upper bounds	Lower bound	Upper bound	Lower bound	Upper bound
Cases prevented	1,065	2,886	388	405	758	1,677	21,147	22,591
DALYs prevented	13,842	37,514	5,047	5,261	9,849	21,799	196,561	209,965
Adjusted DALYs prevented[a]	10,300	27,914	3,755	3,915	7,328	16,220	40,041	42,764
Worthy if the costs lesser than (US$ million)	72.92	197.63	26.59	27.72	51.88	114.84	283.49	302.77
Upfront Cost ($ million)	75.40		103.68–259.20		187.85		37.82–45.88	

According to WHO, an intervention will be cost effective if its CER is less than three times GDP; therefore, the product of its total outcome (adjusted DALYs prevented) and 8,070 (three times the Nigerian GDP) must be higher than its upfront cost for a particular intervention to be considered cost-effective.

The HBV vaccine, but not the postharvest intervention package and NovaSil, is cost-effective at either 59% or 62% HBV attributable fraction on liver cancer. Depending on aflatoxin exposure levels, biocontrol can be either cost-effective or not cost-effective. At the upper bound aflatoxin level, biocontrol is a cost-effective intervention to prevent liver cancer in Nigeria. However, at the lower bound level of aflatoxin, biocontrol is not cost-effective.

4.4.3 Sensitivity Analysis

The sensitivity analyses of these four methods were performed to determine the CERs of these four interventions if their costs and efficacy were changed. We varied the costs by 30–500% of the current costs; and the efficacy from 40% of the original efficacy to their full capacity to completely reduce aflatoxin, aflatoxin bioavailability, or chronic HBV infections.

Table 4-7 through table 4-14 present the CERs of four different types of aflatoxin control interventions at various costs and efficacy. At the lower bound aflatoxin level, either a reduction in cost or an improvement in efficacy is needed for biocontrol to become a worthy intervention. In contrast, even when the cost of the postharvest intervention package was reduced to only 30% of its original cost and its efficacy was improved to completely reduce aflatoxin in groundnuts; this intervention was still not cost-effective. NovaSil needs both cost reductions and efficacy improvements to become a cost-effective intervention. It is worthwhile to note that in this case it does not allow much opportunity for anyone to spend more money to promote or to perform extra activities since it would increase the cost of NovaSil. On the other hand, the HBV vaccine is much more tolerant to cost and efficacy changes compared with the other three interventions in this study.

4.4.3.1 Biocontrol

Table 4-7. Cost effectiveness ratios of biocontrol at the lower bound level of aflatoxin

		Multipliers of original cost							
CER =	7,319	0.3	0.5	1	1.2	1.5	2	3	5
Multipliers of original efficacy	0.4	5,489	9,149	18,298	21,958	27,447	36,596	54,894	91,490
	0.6	3,660	6,099	12,199	14,638	18,298	24,397	36,596	60,993
	0.8	2,745	4,575	9,149	10,979	13,724	18,298	27,447	45,745
	1	2,196	3,660	7,319	8,783	10,979	14,638	21,958	36,596
	1.12	1,961	3,268	6,535	7,842	9,803	13,070	19,605	32,675

Table 4-8. Cost effectiveness ratios of biocontrol at the upper bound level of aflatoxin

		Multipliers of original cost							
CER =	2,702	0.3	0.5	1	1.2	1.5	2	3	5
Multipliers of original efficacy	0.4	2,027	3,378	6,755	8,107	10,133	13,511	20,266	33,777
	0.6	1,351	2,252	4,504	5,404	6,755	9,007	13,511	22,518
	0.8	1,013	1,689	3,378	4,053	5,067	6,755	10,133	16,889
	1	811	1,351	2,702	3,243	4,053	5,404	8,107	13,511
	1.12	724	1,206	2,413	2,895	3,619	4,825	7,238	12,063

4.4.3.2 The postharvest intervention package

Table 4-9. Cost effectiveness ratios of the postharvest intervention package at the lower bound level of aflatoxin

		Multipliers of original cost							
CER =	69,110	0.3	0.5	1	1.2	1.5	2	3	5
Multipliers of original efficacy	0.4	51,832	86,387	172,774	207,329	259,162	345,549	518,323	863,872
	0.6	34,555	57,591	115,183	138,220	172,774	230,366	345,549	575,915
	0.8	25,916	43,194	86,387	103,665	129,581	172,774	259,162	431,936
	1	20,733	34,555	69,110	82,932	103,665	138,220	207,329	345,549
	1.2	17,277	28,796	57,591	69,110	86,387	115,183	172,774	287,957

Table 4-10. Cost effectiveness ratios of the postharvest intervention package at the upper bound level of aflatoxin

		Multipliers of original cost							
CER =	66,142	0.3	0.5	1	1.2	1.5	2	3	5
Multipliers of original efficacy	0.4	49,607	82,678	165,356	198,427	248,034	330,712	496,068	826,781
	0.6	33,071	55,119	110,237	132,285	165,356	220,475	330,712	551,187
	0.8	24,803	41,339	82,678	99,214	124,017	165,356	248,034	413,390
	1	19,843	33,071	66,142	79,371	99,214	132,285	198,427	330,712
	1.2	16,536	27,559	55,119	66,142	82,678	110,237	165,356	275,594

4.4.3.3 NovaSil clay

Table 4-11. Cost effectiveness ratios of NovaSil clay at the lower bound level of aflatoxin

		Multipliers of original cost							
CER =	24,755	0.3	0.5	1	1.2	1.5	2	3	5
Multipliers of original efficacy	0.4	18,566	30,943	61,887	74,264	92,830	123,774	185,660	309,434
	0.6	12,377	20,629	41,258	49,509	61,887	82,516	123,774	206,289
	0.8	9,283	15,472	30,943	37,132	46,415	61,887	92,830	154,717
	1	7,426	12,377	24,755	29,706	37,132	49,509	74,264	123,774
	1.2	6,189	10,314	20,629	24,755	30,943	41,258	61,887	103,145
	1.4	5,305	8,841	17,682	21,218	26,523	35,364	53,046	88,410
	1.6	4,642	7,736	15,472	18,566	23,208	30,943	46,415	77,359

Table 4-12. Cost effectiveness ratios of NovaSil clay at the upper bound level of aflatoxin

		Multipliers of original cost							
CER =	11,177	0.3	0.5	1	1.2	1.5	2	3	5
Multipliers of original efficacy	0.4	8,383	13,971	27,943	33,531	41,914	55,885	83,828	139,713
	0.6	5,589	9,314	18,628	22,354	27,943	37,257	55,885	93,142
	0.8	4,191	6,986	13,971	16,766	20,957	27,943	41,914	69,857
	1	3,353	5,589	11,177	13,412	16,766	22,354	33,531	55,885
	1.2	2,794	4,657	9,314	11,177	13,971	18,628	27,943	46,571
	1.4	2,395	3,992	7,984	9,580	11,975	15,967	23,951	39,918
	1.6	2,096	3,493	6,986	8,383	10,479	13,971	20,957	34,928

4.4.3.4 Hepatitis B vaccine

Table 4-13. Cost effectiveness ratios of HBV vaccine at 59% attributable fraction of HBV on liver cancer

		Multipliers of original cost							
CER =	1,146	0.3	0.5	1	1.2	1.5	2	3	5
Multipliers of original efficacy	0.4	859	1,432	2,865	3,437	4,297	5,729	8,594	14,323
	0.6	573	955	1,910	2,292	2,865	3,819	5,729	9,548
	0.8	430	716	1,432	1,719	2,148	2,865	4,297	7,161
	1	344	573	1,146	1,375	1,719	2,292	3,437	5,729
	1.05	327	546	1,091	1,310	1,637	2,183	3,274	5,456

Table 4-14. Cost effectiveness ratios of HBV vaccine at 62% attributable fraction of HBV on liver cancer

		Multipliers of original cost							
CER =	1,073	0.3	0.5	1	1.2	1.5	2	3	5
Multipliers of original efficacy	0.4	805	1,341	2,682	3,218	4,023	5,363	8,045	13,409
	0.6	536	894	1,788	2,145	2,682	3,576	5,363	8,939
	0.8	402	670	1,341	1,609	2,011	2,682	4,023	6,704
	1	322	536	1,073	1,287	1,609	2,145	3,218	5,363
	1.05	306	511	1,022	1,226	1,532	2,043	3,065	5,108

4.5 DISCUSSION

In our previous chapter, we have shown that the HBV vaccine is an effective intervention to control liver cancer in Nigeria. In this study, we show that the HBV vaccine is a cost-effective intervention based on WHO criteria. Aside from preventing liver cancer developments, an immunization against the HBV also reduces the chance of acute infections by the HBV and reduces other consequences of chronic HBV infections, including cirrhosis. Therefore, total benefits of the HBV vaccine could be much more than the numbers presented in this study.

None of the aflatoxin control interventions selected in our studies show superior efficacy to prevent liver cancer in Nigeria over the HBV vaccine. Though at the upper bound level of aflatoxin, biocontrol can be a cost-effective intervention; the other two interventions are less likely to be cost-effective. At the lower bound aflatoxin level, reducing the costs or improving the efficacy will increase the chance of biocontrol to be a cost-effective intervention. In order to be worthy, huge cost reductions (<30% of the original cost) together with efficacy improvements are needed for the postharvest intervention package. Because the cost of wooden pallets–used for uplifting stored groundnuts bags– was the main cost of this package, using readily available materials in households would greatly reduce the cost of the intervention. Since a Nigerian consumes a much larger amount of maize than groundnuts; therefore, one of the strategies to improve the efficacy of the postharvest intervention package is to apply this package to maize.

To become a cost-effective intervention, NovaSil requires both cost reductions and efficacy improvements in most of cases. One possible way to reduce the cost and improve its efficacy is by targeting this intervention to high risk populations, such as individuals who are HBV-positive.

Though some aflatoxin control interventions are not worthy to prevent liver cancer in Nigeria if preventing liver cancer development is the only outcome of interest, it can be misguided for anyone to inscribe these interventions to be non-worthy without taking into account of their other benefits, such as preventing growth impairment induced by aflatoxin. In the next two chapters, we reviewed the associations between aflatoxin and growth performances in animals and humans and performed the cost-effective assessment to determine whether the cost-effectiveness results change if benefits from preventing growth impairment are included.

5.0 AFLATOXINS AND GROWTH IMPAIRMENT: A REVIEW

Pornsri Khlangwiset[1], Gordon S. Shephard[2], Felicia Wu[1*]

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Pornsri Khlangwiset[1], Gordon S. Shephard[2], Felicia Wu[1]

[1]Department of Environmental and Occupational Health, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA, USA

[2]PROMEC Unit, Medical Research Council, P.O. Box 19070 Tygerberg, South Africa

5.1 ABSTRACT

Aflatoxins, fungal toxins produced by *Aspergillus flavus* and *A. parasiticus* in a variety of food crops, are well-known as potent human hepatocarcinogens. Relatively less highlighted in the literature is the association between aflatoxin and growth impairment in children. Foodborne aflatoxin exposure, especially through maize and groundnuts, is common in much of Africa and Asia: areas where childhood stunting and underweight are also common, due to a variety of possibly interacting factors such as enteric diseases, socioeconomic status, and sub-optimal nutrition. The effects of aflatoxin on growth impairment in animals and human children are reviewed, including studies that assess aflatoxin exposure *in utero* and through breastfeeding. Childhood weaning diets in various regions of the world are briefly discussed. Our review suggests that aflatoxin exposure and its association with growth impairment in children could contribute a significant public health burden in less developed countries.

5.2 INTRODUCTION

5.2.1 Aflatoxins

Much of the policy attention surrounding *aflatoxin*, a common contaminant in the global food supply, has focused on its role in inducing liver cancer in humans (132, 223-225); with little or no attention devoted to the role of aflatoxin in growth impairment. Among other reasons, it is because the weight of evidence linking aflatoxin to human growth impairment has historically been much weaker than that linking aflatoxin to human liver cancer. However, animal studies over the last several decades have demonstrated a significant association between aflatoxin and growth impairment; and, especially in the last decade, epidemiological studies have emerged suggesting similar effects in human children. Validation of serum- and urine-based biomarkers of aflatoxin exposure and effect in the last two decades has greatly assisted these epidemiological

studies (Groopman et al. 2008). In this paper, we review the literature associating aflatoxin with growth impairment in both animals and humans.

Aflatoxins are secondary metabolites of the fungi *Aspergillus flavus*, *A. parasiticus*, and occasionally other *Aspergillus* species. These fungal species are prevalent in food crops, particularly maize, groundnuts, oilseeds, and tree nuts, in tropical and subtropical regions worldwide. Factors that influence whether these fungi produce aflatoxin include drought stress and rainfall, adaptation ability of crop genotype for its climate, insect damage, and agricultural practices (226). These fungi can also produce aflatoxin in postharvest conditions: food storage, transportation, and processing. Maize and groundnuts are the major sources of aflatoxin exposure in humans (the number of exposed persons exceeding several billion) because of the high consumption rates of these foods worldwide and their susceptibility to *Aspergillus* infection (6).

Aflatoxin B_1 (AFB_1), the most toxic form of the aflatoxins, is the most potent naturally occurring chemical liver carcinogen known. For people who are chronically infected with hepatitis B virus (HBV; common in China and Africa), aflatoxin consumption synergistically increases the risk of hepatocellular carcinoma (HCC; liver cancer) compared with either exposure alone (121). Acute aflatoxicosis, characterized by hemorrhage, acute liver damage, edema, and death, can result from extremely high doses of aflatoxin in the diet (227). In 2004 and 2005, hundreds of acute aflatoxicosis cases in Kenya and 125 deaths were associated with the consumption of contaminated home-grown maize (6). Aflatoxin exposure has also been associated with immunotoxicity in humans (10, 20, 22, 23); and, as this review highlights, with stunted growth and other indicators of growth impairment in children.

5.2.2 Growth impairment and global burden of disease

Childhood growth performance is usually measured by one or more of three indicators: height for age, weight for age, and weight for height. Based on WHO definitions, children whose heights for ages, weights for ages, and weights for heights are two standard errors or more below WHO growth standards (z score ≤ -2) are considered to be *stunted*, *underweight*, and *wasted*, respectively (228). Wasting is an indicator of deficits in tissue and fat mass, which may be caused by acute malnutrition; while stunting is regarded as the indicator of chronic malnutrition.

The prevalence of severe wasting decreases by 24 months of age, while stunting prevalence increases by age and reaches a plateau at 24–36 months (229, 230).

Stunting is a widely used indicator of chronic malnutrition in early childhood, including malnutrition during fetal development due to poor maternal nutrition. Children are considered stunting if their height-for-age z-score (HAZ) is -2 or lower. Once established, stunting and its effects usually last for years. Children who are stunted often develop long-term developmental and cognitive problems, and are more vulnerable to infectious diseases (231). In one study, Filipino children aged between 8 and 11 years old who were stunted as two-year-olds had significantly lower test scores than non-stunted children later in life; as well as delays in school enrollment, increased school absences, and repetition of school years (232).

An average disability weight, a weight factor that reflects severity of a disease, of 0.002 for each stunting case, based on a 0–1 scale assigned by the World Health Organization (208), is relatively low compared with other conditions, diseases, or injuries. This is because the associated risk factors of stunting (increased susceptibility to infectious disease, cognitive impairment) are not included in the estimation of the disability weight of stunting. However, stunting may still cause a high global burden of disease because of its prevalence, as well as its associated risk factors, and hence deserves public health attention. In 2004, an estimated 182.7 million children in developing countries were considered to be stunted. 70% of these stunted children live in South and Southeast Asia and sub-Saharan Africa (208). Globally, 21% of deaths and disability adjusted life years (DALYs) in children aged five years and under are estimated to be attributed to stunting, severe wasting and intra-uterine growth restriction (229).

Table 5-1 lists socioeconomic characteristics of selected nations worldwide, and estimated dietary aflatoxin exposure and proportion of stunted children. Though the relationship is not consistent, it appears that in general, the proportion of childhood stunting is directly correlated with proportion of population living below the national poverty line, and inversely correlated with GDP per capita. As is the case with HCC, childhood stunting is prominent in world regions where foodborne aflatoxin exposure is high: South and East Asia, and sub-Saharan Africa.

Table 5-1. Economic, aflatoxin exposure, and health characteristics of selected nations.

Country	% population living below national poverty line (233)	GDP per capita, 2010 USD (PPP) (211)	Aflatoxin exposure, ng/kgBW/day (142)	% stunted children (233)
Argentina	NA	15,030	0–4	8
China	5	7,240	17–37	22
France	NA	34,250	0.3–1.3	NA
The Gambia	58	1,479	4–115	28
India	29	3,176	4–100	48
Kenya	52	1,783	3.5–133	36
Nigeria	34	2,357	139–227	43
Philippines	37	3,604	44–54	34
Spain	NA	29,649	0.3–1.3	NA
Tanzania	36	1,484	0.02–50	44
Thailand	13	8,479	53–73	16
USA	NA	47,702	0.26	4

Note: . GDP = gross domestic product per capita, NA = not available, PPP = purchasing power parity

Underweight children are significantly more at risk of death from diseases including diarrhea, pneumonia, malaria, and measles. It has been estimated that children with a weight-for-age z-score (WAZ) of –1 to –2 are twice as likely to die from diarrheal diseases compared with children of normal weight, while children with WAZ from –2 to –3 are five times as likely to die. Additionally, 52% of pneumonia deaths in children age 5 and under are associated with low body weight (234). Although the prevalence of underweight is expected to decrease from 26.5% in 1990 to 17.6% in 2015, this decrease would not be uniform across the world. In Asia and Latin America, childhood underweight is expected to decrease by about 50%, while in Africa, underweight prevalence may even increase by 2–3% compared with 1990 (235). WHO does not assign a s pecific disability weight to childhood underweight; however, low birth weight is assigned a disability weight of 0.106 per case (208).

Wasting in children (weight-for-height z-score, or WHZ, is -2 or lower) is believed to be a condition related to acute malnutrition (236, 237), either from insufficient food intake or infectious diseases. Immune system impairment in wasted children makes them more susceptible

to infections (238). As a result, wasting increases the risk of death in children with this condition (239). W HO assigns a disability weight of 0.053 per wasting case. In 2004, the global prevalence of wasting in children aged 5 years and under was about 56.2 million (208).

This manuscript reviews the evidence linking aflatoxin exposure to growth impairment in animals and in human children. We first review the literature on animal studies over the past 50 years in which associations were found between aflatoxin exposure and reduced feed intake, reduced weight gain, and other measures of growth impairment in animals. Then we describe the studies that show evidence of aflatoxin exposure in children in various parts of the world, review a previously examined association between aflatoxin exposure and kwashiorkor (a disease of protein energy malnutrition), and discuss the studies that link aflatoxin exposure with stunting, underweight, and wasting in children. We describe weaning foods in various cultures worldwide, and end with a discussion of possible mechanisms by which aflatoxin may result in growth impairment in animals and humans.

5.3 AFLATOXIN AND GROWTH IMPAIRMENT IN ANIMALS

The adverse effects of aflatoxin on various indicators of growth performance have been demonstrated in multiple animal species over the last five decades. Reduced feed intake and subsequent weight gain reduction in animals exposed to aflatoxin have been reported in mule ducklings (240), mice (241), Japanese quail (242), Cherry Valley commercial ducks (243), chickens (71, 244-252), turkeys (246), pigs (21, 253-262), Nile tilapia (263), and channel catfish (264).

In addition, increases in feed conversion ratio (FCR)–defined here as the mass of food intake per unit weight gain by the animal−were prominent in the animals dosed with aflatoxin (244, 246, 253, 254, 258, 265, 266). Aflatoxin at relatively lower doses (100–300 μg/kg) did not affect six-month old steer; but their weight, feed intake, and feed efficiency were adversely affected at higher doses (700–1,000 μg/kg) (265). While channel catfish and rainbow trout were not affected by 2,154 μg/kg of purified aflatoxin in feed diet in terms of growth rate, FCR, or liver lesions (264), Nile tilapia showed significant reduction in weight and feed consumption

when exposed to 1.8 mg/kg diet (1,800 μg/kg) or higher doses of aflatoxin for 25 days (263). Taken together, these studies suggest that aside from reducing feed intake and weight gain, feed conversion efficiencies across multiple animal species were also reduced by aflatoxin exposure in the diet.

Several studies observed effects of aflatoxin on growth in baby animals as a result of *in utero* exposure through maternal feed. The effect of aflatoxin on offspring was reported by Butler and Wigglesworth in 1966, who observed growth retardation in rat pups whose mothers were fed high doses of aflatoxin during late pregnancy (267). The fetuses of hamsters administered aflatoxin intra-peritoneally at doses of 4 mg/kgBW or 6 mg/kgBW on days 8 and 9 of pregnancy experienced growth retardation, compared with controls, but there were no significant differences in malformations between the two groups (268). Kihara et al. (2000) reported lower mean body weight, delayed physical and behavioral development in the pre-weaning phase, and disability of locomotor coordination and impaired avoidance performance in the post-weaning periods, in rat pups whose mothers were exposed to 0.3 mg/kg/day of aflatoxin subcutaneously during pregnancy. Moreover, they found that numbers of live births were affected by aflatoxin exposure during prenatal periods (269). Maternal exposure to AFB_1, not AFG_1, decreased body weights of piglets significantly (270). While aflatoxin did not affect average egg production, feed conversion, and body weight, it affected feed consumption and egg weight of laying Japanese quail (271).

Table 5-2 and table 5-3 contain summaries of animal studies showing an association between aflatoxin exposure and growth reduction, in animals that directly consume aflatoxin (table 5-2) and *in utero*: in baby animals whose mothers were exposed to aflatoxin during pregnancy (table 5-3).

Table 5-2. Animal studies of the effects of aflatoxin exposure on animal growth.

Animal	Aflatoxin dose and duration of experiment	Results	Study
Pigs (n=50)	0 (A), 0.2 (B), 0.7 (C),1.1 (D) mg/kg feed (16 weeks)	No significant difference in body weight between groups. Increase in FCR [4.53 (A), 4.55 (B), 4.67 (C), 4.76 (D)] ($p<0.05$)	Armbrecht et al. (1971)
Pigs (n=60)	0 (A), 1.0 (B), 2.0 (C), 4.0 (D) mg/kg feed (13 wks)	Increase in FCR [3.14 (A), 3.82 (B), 4.13 (C), NA (D)[a]] ($p<0.001$)	Armbrecht et al. (1971)
Weanling pigs (n=110)	<2 (A), <8 (B), 51 (C), 105 (D), 233 (E) µg/kg feed (120 days)	No significant effect on weight gain or feed conversion	Keyl and Booth (1971)
Weanling pigs (n=110)	<6 (A), 450 (B), 615 (C), 810 (D) µg/kg feed (120 days)	Decrease in ADG at the dose of 615 and 810 µg/kg feed [0.71 kg (A), 0.60 kg (C), 0.47 kg (D)] ($p<0.05$)	Keyl and Booth (1971)
Young cross-bred steers (n=50)	0(A), 100 (B), 300 (C), 700 (D), 1 000 (E) µg/kg (133 days)	Decrease in ADG at 700, and 1 000 µg/kg ($p<0.01$) [1.14 kg (A), 0.86 kg (D), and 0.79 kg (E)] Increase in FCR at 700, and 1 0 00 µg/kg ($p<0.01$) [5.7 (A), 6.4 (D), 6.6 (E)]	Keyl and Booth (1971)
30-day-old Sprague-Dawley rats (n=24)	0 (A), DMSO (B), 5 mg/kgBW of AFB_1 in DMSO, 7 mg/kgBW of AFB_1 in DMSO (IP single dose in 76 hrs)	Weight gain in 0, and DMSO groups [13g/rat (A), 15 g/rat (B)] Weight lost in 5 and 7 mg/kgBW of AFB_1 in DMSO groups [1 g/rat (C), 8g/rat (D)]	Doyle et al. (1977)
30-day-old Sprague-Dawley rats	DMSO, 10 mg/kgBW of AFB1 in DMSO (IP single dose in 54 hours)	Weight gain in DMSO group [8g/rat] Weight lost in 10 mg/kgBW of AFB1 in DMSO group [20 g/rat (C)]	Doyle et al. (1977)
Chickens (n>900)	0 (A), 0.3 (B),1.25(C), 2.0 (D) mg/kg (28 days)	Decrease in body weight and food intake Increase in FCR ($p<0.001$)	Bryden et al. (1979)

Table 5-2 (continued)

Animal	Aflatoxin dose and duration of experiment	Results	Study
Broiler chicks (n=40 –48)	0 (A), 5 mg/kg feed aflatoxin (B), exercise (C), 5 mg/kg feed aflatoxin +exercise (D) (24 days)	Decrease in body weight in aflatoxin treated group which can be partially improved by exercise [557.6±9.3 g (A), 542.7±9.0 g (B), 366.8±7.4 g (C), 412.5±7.4 g (D)]. Increase in FCR in aflatoxin treated group [1.54 (A), 1.89 (C)]	Randall and Bird (1979)
Layer type chicks (n=40 –48)	0 (A), 5 mg/kg feed aflatoxin (B), exercise (C), 5 mg/kg feed aflatoxin +exercise (D) (39 days)	Decrease in body weight in aflatoxin treated group which can be partially improved by exercise. [469.5±9.9 g (A), 469.5±9.9 g (B), 370.8±20.2 g (C), 384.1±14.4 g (D)]. Increase in FCR in aflatoxin treated group [1.59 (A), 1.75 (C)].	Randall and Bird (1979)
Broiler chicks (n=40 –48)	0 (A), 5 mg/kg feed aflatoxin (B), exercise (C), 5 mg/kg feed aflatoxin +exercise (D)	Decrease in body weight in aflatoxin treated group which can be partially improved by exercise. [510.5±12.5 g (A), 502.0±12.0 g (B), 414.9±19.8 g (C), 434.0±8.1 g (D)]. No difference in FCR.	Randall and Bird (1979)
Pigs (n=32: 8 for each of 4 g roups of pigs)	20(A), 385 (B), 750 (C), and 1 480 (D) µg/kg (control: 20 µg/kg group)	Decrease in ADG (dose-related) [0.77 kg (A), 0.67 kg (B), 0.57 kg (C), 0.41 kg (D)]; and ADFI [2.87 kg (A), 2.53 kg (B), 2.15 kg (C), 1.61 kg (D)] ($p<0.05$). Increase in FCR in the 1 480 µg/kg treated group [3.74 (A), 3.97 (D)] ($p<0.05$)	Southern and Clawson (1979)
Broiler chickens (n=75)	0 (A), 0.075 (B), 0.225 (C), and 0.675(D) mg /kg feed (7 weeks)	Decrease in body weight in all aflatoxin-treated groups [2 256±21 g (A), 2 098±26 g (B), 1 989±20 g (C), 2 047±24 g (D)] ($p<0.05$)	Doerr et al. (1983)

Table 5-2 (continued)

Animal	Aflatoxin dose and duration of experiment	Results	Study
Broiler chickens (n=75)	0 (A), 0.3(B), 0.9 (C), and 2.7 (D) mg /kg feed (7 weeks)	Decrease in body weight in only 2.7 mg of aflatoxin per kg feed-group [2 024±30 g (A), 1 671±36 g (D)] ($p<0.05$)	Doerr et al. (1983)
1-day-old broilers (n=70)	0 (A), .625 (B), 1.25 (C), 2.5 (D), 5.0 (E), and 10.0 (F) mg/kg feed (3 weeks)	Aflatoxin dose-related decrease in body weight at the dose 1.25 µg /g and higher [511±32 g (A), 463±16 g (D), 386±25 g (E), 286±13 g (F)] and feed consumption [851±52 g (A), 773±50 g (D), 703±55 g (E) 734±14 g (F)] ($p<0.05$)	Huff (1980)
14-day-old turkeys (n=200)	0 (A), 100 (B), 200 (C), 400 (D), or 800 (E) µg/kg (AFB_1) (35 days)	Decrease in percent weight gain at the dose of 400 µg/kg and higher [averaged 5- week percent weight gain :48.2% (A), 33.2% (D), 19.7%(E)]. Increase in FCR at the two highest doses [FCR averaged in 5-week: 1.81 (A), 1.89 (D), 2.28 (E)] ($p<0.05$)	Giambrone et al. (1985)
14 days old broiler chickens (n=200)	0 (A), 100 (B), 200 (C), 400 (D), or 800 (E) µg/kg (AFB_1) (35 days)	No significant difference in weight gain ($p>0.05$) Increase in FCR at the dose of 800 µg/kg [FCR: 2.02 (A), 2.11 (E)]	Giambrone et al. (1985)
105 days old chicks (n=120)	0 (A) , 2.5 (B), 5.0 (C), and 10.0 (D) mg/kg feed (4 weeks)	Aflatoxin dose related decrease in body weight ($p<0.05$) [1.85±0.03 kg (A), 1.57±0.05 g (B), 1.51±0.04 kg (C), 1.47±0.03 g (D)]	Shukla and Pachauri (1985)
Male broiler chicks (n=180)	0 (A) , 2.5 mg/kg of aflatoxin (B) , and 2.5 mg/kg of aflatoxin + 16 mg/kg of deoxynivalinol (C) (3 weeks)	Decrease in body weight [626±11 g (A), 521±12 g (B), 488±9 g (C)], weight gain [490±10 g (A), 397±10 g (B), 365±8 g (C)], protein serum levels [2.9±0.1g/100ml (A), 2.0±0.1 g/100ml (B), and 2.1±0.1 g/100 ml (C)] ($p<0.05$)	Huff et al. (1986)

Table 5-2 (continued)

Animal	Aflatoxin dose and duration of experiment	Results	Study
5–6 weeks old pigs (n= 30: 10 each in control, 300 and 500 µg/kg groups)	0, 300 and 500 µg /kg of feed (10 weeks)	Decrease in weight gain in both aflatoxin treated groups up to 2 kg in 10-week period and feed consumption in high dose group compared with controls ($p<0.01$)	Panangala et al. (1986)
1-day-old broilers and layer chicks (n= 40 each)	0 (A), 1 (B), 4 (C) mg/kg feed (4 weeks)	Aflatoxin dose dependent decrease in body weights ($p<0.05$). Broilers: [332±17.81 g (A), 254±14.35 g (B), 239±13.50 g (C)], Layer chicks : [158±3.6 g (A), 139±4.41 g (B), 126±5.82 g (C)]	Ram et al. (1988)
7- week- old Pigs (n=15)	0 (A), 2.0 mg of aflatoxin (B), , 2.0 mg of ochratoxin (C), and 2.0 mg of aflatoxin + 2.0 mg ochratoxin (D) /kg of feed (28 days)	Decrease in body weight gain in all aflatoxin –treated groups [18.2±0.9 kg (A), 13.5±0.8 kg (B), 13.8±1.0 kg (C), 8.8±0.9 kg (D)] ($p<0.05$)	Harvey et al. (1989)
Channel catfish (n=450)	0, 100, 404, 2154, or 10 000 µg/kg (10 weeks)	Decrease in weight gain in the 10 000 µg/kg group by 24% compared with the control ($p<0.05$)[weight gain per fish in the highest dosed group = 60 g compared with 80 g/fish in the control group]	Jantrarotai and Lovell (1990)
Weanling swine (n=90).	0 (A), 420 (B) , 840 (C) µg/kg feed (49 days)	Decrease in ADG [0.52 kg (A), 0.46 kg (B), 0.28 kg C)]; ADFI [1.13 kg (A), 0.95 kg (B), 0.67 kg (C)] Increase in FCR [1.72 (A), 1.92 (B), 2.70 (C)] (linear $p<0.01$, and quadratic, $p< 0.05$)	Lindemann et al. (1993)
Weanling swine (n=63)	0 (A), 800 (B) µg/kg feed (42 days)	Decrease in ADG [0.64 kg (A), 0.41 kg (B)], ADFI [1.32 (A), 0.82 kg (B)]	Lindemann et al. (1993)
Weanling pigs (n=96)	0 (A), 922 (B) µg/kg feed (6 weeks)	Decrease in ADG [0.505 kg (A), 0.392 kg (B)] and ADFI [1.10 kg (A), 0.88 kg(B)] ($p<0.01$)	Schell et al., (1993a)

Table 5-2 (continued)

Animal	Aflatoxin dose and duration of experiment	Results	Study
Weaned Pigs (n=54)	0 (A), 800 (B) µg/kg feed (4 weeks)	Decrease in ADG [0.64 kg (A), 0.48 kg (B)] ($p<0.05$), ADFI [1.32 kg (A), 1.0 kg (B)] ($p>0.05$) Increase in FCR [2.08 (A), 2.43 (B)] ($p<0.05$)	Schell et al., (1993b)
Weaned Pigs (n=81)	0 (A), 500 (B) µg/kg feed (5 weeks)	Decrease in ADG [0.66 kg (A), 0.46 kg (B)], ADFI [1.41 kg (A), 0.97 kg (B)] ($p<0.05$)	Schell et al. (1993b)
Weaned Pigs (n=63)	0 (A), 800 (B) µg/kg feed (4 weeks)	Decrease in ADG [0.63 kg (A), 0.52 kg (B)] ($p<0.05$), ADFI [1.29 kg (A), 1.02 kg (B)] ($p<0.01$)	Schell et al. (1993b)
Nile tilapia (n= 160)	0 (A), 0.94 (B), 1.88 (C), 0.375(D), 0.752 (E), 1.50 (F), 3.0 (G) mg/kg diet (25 days following with basal diet for 50 days)	Decrease in ADG, and ADFI, but not FCR in 1.88 mg/kg group and higher ADG: [10.87–11.30 g (A), 7.28 g (C), 7.10g (D), 4.78 g (E), 3.25 g (F), 3.66 g (G)] ($p<0.01$) ADFI: : [0.143-0.160 g (A), 0.115 g (C), 0.116 g (D), 0.711 g (E), 0.052 g (F), 0.048 g (G)] ($p<0.01$)	Chávez-Sánchez et al. (1994)
Lambs (n=44)	0 mg aflatoxin in soybean meal (A), 0 mg aflatoxin in fish meal (B), 2.5 mg/kg diet soybean meal (C) or 2.5 mg/kg diet fish meal (D) (35 days, followed by 32 days wash out period)	Decrease in feed intake, daily gain, in aflatoxin-fed lambs ($p<0.05$) during treatment period and wash out period. ADG: 0.53 kg (A), 0.24 kg (C), 0.50 kg (B), 0.05 kg (D). ADFI: 4.19 kg (A), 2.74 kg (C), 4.05 kg (B), 1.70 kg (D) Increase in FCR in aflatoxin-fed lambs ($p<0.05$) FCR: 7.6 (A), 11.2 (C), 7.6 (B), –45.5 (D)	Edrington et al. (1994)
Growing barrows (n=40)	0 (A), 3 (B) mg/kg feed[b] (28 days)	Decrease in weight gain [19.1±0.73 kg (A), 10.7±1.06 kg (B)] ($p<0.05$)	Harvey et al. (1994)

Table 5-2 (continued)

Animal	Aflatoxin dose and duration of experiment	Results	Study
Pigs (n=27)	0 (A), 2.5 mg AF /kg feed (B), 2.5 mg of AF/kg feed + 2400 IU tocopherol (C) (32 days)	Decrease in body weight [38.4±3.9 kg (A), 22.0±2.0 kg (B), and 23.5±3.0 kg (C)], and feed consumption [138±20.0 kg (A), 41±4.5 kg (B), and 45±2.0 kg (C)] ($p<0.05$)	Harvey et al. (1995a)
Pigs (n=18)	0 (A), 2.5 (B), 2.5 mg of aflatoxin plus 100 mg of fumonisin B_1/kg of feed (C) (35 days)	Decrease in body weight [49.2 kg (A), 33.2 kg (B) , 23.9 kg (C)], weight gain [31.6 kg (A), 15.8 kg (B) , 6.3 kg (C)] and feed consumption per pen [153.7 kg (A), 89.0 kg (B) , and 42.7 kg (C)]	Harvey et al. (1995b)
1- day-old broiler chicks (n=40)	0 (A), 0.5(B) mg/kg feed (32 days)	Decrease in body weight [246.32±2.14g (A), 140.79±1.31 g (B)], percentage weight gain [100% (A), 57% (B)], and total feed consumption [691.0 g (A), 590.0 g (B)]($p<0.01$)	Prabaharan et al. (1999)
Mule ducklings (n=320)	0 (A),200 (B) µg/kg feed (3- week)	Decrease in daily feed intake [37.74±2.57g (A), 31.99±0.33 g (B)], and average daily weight gain [25.29±1.23 g (A), 21.24±1.25 g (B)] ($p<0.05$).	Cheng et al. (2001)
4-week-old weanling piglets (n=36)	0 (A), 240 , 480 µg/kg feed (30 days)	Decrease in ADG [489±18g (A), 453±12g (B), 326±17g (C)] ($p<0.05$)	Marin et al. (2002)
7-week-old Japanese quail (n =256)	0(A), 25 (B), 50 (C), or 100(D) µg /kg feed (AFB_1) (168 days)	Decrease in ADFI groups exposed to 50 and 100 µg AFB_1/kg. [28.69±2.17g (A), 27.57±1.81g (C), 27.76±1.85g (D)]($p<0.05$) No effect: Average egg production, feed use, and body weights ($p> 0.05$)	Oliveira et al. (2002)

Table 5-2 (continued)

Animal	Aflatoxin dose and duration of experiment	Results	Study
6-week-old male Swiss albino mice (n=70)	0 µg AFB_1 + 5% protein diet (A), 0 µg AFB_1 + 20% protein diet (B), 0.5 µg AFB_1/day + 5% protein diet (C), 0.5 µg AFB_1/day + 20% protein diet (D) (7 weeks).	Decrease in percent weight gain ($p<0.001$) in aflatoxin/normal protein- fed mice (D) compared with nonaflatoxin/normal protein treated (B), but contrast to the low diet groups [4% (A), 18.2% (B), 7.2%(C) and 12.2% (D)]. No significant difference of total protein and albumin levels between aflatoxin treated and non-aflatoxin treated mice ($p > 0.05$)	Kocabas et al. (2003)
1-day-old broiler chicks (n=48)	0 (A), 5 (B) mg/kg of AFB_1 in feed (3 weeks)	Decrease in weight gain [866±12.7 g (A), 699±38.5 g (B)] ($p<0.05$) and feed intake [1369±45.7 g (A), 957±183.5 g (B)] ($p<0.05$)]. No change in FCR.	Pimpukdee et al. (2004)
Cherry Valley commercial ducks (n=90)	0 (A), 20 (B) or 40 (C) µg/kg AFB_1-contaminated rice (6 weeks)	Decrease in ADG in 20 a nd 40 µg/kg AF-treated groups [48.21±2.5 g (A), 42.52±2.5 g (B), and 37.44±2.7 g (C)]; and feed intake in the high dose group [142.20±4.6 g (A), 130.28±3.5 g]. Increase in FCR in both aflatoxin-treated groups [2.95±0.02 (A), 3.31±0.04 (B), 3.48±0.04 (C)] ($p<0.05$)	Han et al. (2008)

Note: Letters (e.g., A, B, C, D) represent different dose groups. [a] Eight of 15 pigs in 4.0 mg/kg feed had severe morbid, hemorrhaged or died. [b]Aflatoxin content consists of 79% AFB_1,16% AFG_1, 4% AFB_2, and 1% AFG_2, ADG =average daily gain, ADFI = average daily feed intake, DMSO = dimethylsulfoxide, FCR= feed conversion ratio, IP= intraperitoneal, NA = not available, , only animals referred in "aflatoxin dose and duration" were counted for sample size

In summary, 30 animal studies are documented here (table 2). Twenty-nine of 30 studies indicated that animals treated with aflatoxin showed reduced weight gain or some deviant signs

such as reduced feed intake or increased feed conversion ratio. Only one study reported non-significant effect in either body weight or feed conversion.

Table 5-3. Studies showing an association between aflatoxin exposure *in utero* and reduced growth in baby animals.

Animal	Aflatoxin dose and duration of experiment	Results	Study
Rats: (n= 25)	0 (n=NA), 1 mg crystalline aflatoxin in 0.1–0.2 ml of dimethylformamide (dosed orally as single dose at d 6 (n=6), d6–d12 (n=10), and d16 of gestation (n=9))	Decrease in fetal weight ($p < 0.01$) in offspring rats born from mothers given aflatoxin in late pregnancy [4.75± 0.059 g (untreated), 3.81±0.066 g (d-16 group)]	Butler and Wigglesworth (1966)
Hamster (n= 40: 16 control, 24 tested)	4 or 6 mg/kg of crystalline AFB1 by i.p. injection on days 8 and 9 of pregnancy	Decrease in fetal growth in offspring hamster by 0.5–0.7 centimeter differences in length ($p<0.01$)	Schmidt and Panciera (1980)
2 –3 years old sows and their piglets (n=24)	0 (A), 800 µg/kg AFG_1(B), 800 µg/kg AFB_1(C), 800 µg/kg AFG_1+800µg/kg AFB_1 (D) (d60 of pregnancy to d28 of lactation)	Decrease in piglets' body weight in AFB_1 –treated groups, but not in AFG_1–treated group[: [6.51±0.42 g (A), 5.66±0.39 g (B), 5.32±0.63 g (C), 5.25±0.44 g (C)] ($p<0.05$, and < 0.005 for C and D groups, respectively	Mocchegiani et al. (1998)

Table 5-3 (continued)

Animal	Aflatoxin dose and duration of experiment	Results	Study
Rat, pregnant and offspring (n=30, 10 of each three groups)	0 (A), 0.3 mg/kg/day AFB_1 dissolved in dimethylsulfoxide subcutaneously on days 11–14 (B) or 15–18 (C) of gestation	Decrease in mean birth weights in both male and female offspring treated with AFB_1 [Male: 5.7±0.1 (A), 5.1±0.1 (B), 4.7±0.3 (C); Female: 5.4±0.1 (A), 4.9±0.1 (B), 4.7±0.3 (C)] ($p<0.05$), decrease in number of live births in the group exposed to AFB_1 on d15 –18 [15.2±0.5 (A), 12.9±0.9] , delayed physical development, and delayed behavioral developments during pre-weaning period, impaired locomotor coordination and deficits in avoidance performance in postweaning period	Kihara et al., 2000
7 weeks old Japanese quail (n =256)	0(A), 25 (B), 50 (C), or 100(D) µg /kg feed (AFB_1) (168 days)	Decrease in egg weight ($p < 0.05$) in groups exposed to 50 and 100 µg AFB_1/kg [10.67±0.24 (A), 10.53±0.21 (C), 10.51±0.21 (D)]. Increase : Percent shell of total egg weight ($p< 0.05$) in 100 µg AFB_1/kg fed group	Oliveira et al. (2002)

Note: Only animals referred in "aflatoxin dose and duration" were counted for sample size

Table 5-3 lists five animal studies which show the association between in utero aflatoxin exposure and growth parameters in baby animals. All five studies reported either the reduced fetal weights/ egg weights or fetal lengths of the offspring animals.

5.4 AFLATOXIN AND GROWTH IMPAIRMENT IN HUMANS

5.4.1 Aflatoxin exposure *in utero* and in early childhood

Exposure to aflatoxin begins early in the lives of many children worldwide. Children may be exposed to aflatoxin through maternal food intake *in utero*, through breastfeeding, and through weaning and post-weaning foods; particularly where maize and groundnuts are dietary staples. Aflatoxin exposure increases most dramatically after children are weaned from breastfeeding (272). However, even *in utero* exposures can have a significant effect on faltering in infant growth (32).

Detection of aflatoxins and aflatoxin-albumin adducts (AF-alb) in the cord blood of babies in various countries confirm that children are exposed to aflatoxin and/or its metabolites *in utero*. In a Taiwanese study, 11 of 120 placentas were found to contain aflatoxin DNA adduct levels ranging from 0.6 to 6.3 μmol/mol DNA. In the same study, aflatoxin DNA adducts were detected in 6 of 56 cord blood samples in the range of 1.4–2.7 μmol/mol DNA (273). Aflatoxin M_1 (AFM_1), a metabolite of AFB_1, was detected in 68% (113/166) and 67% (111/ 166) of maternal blood and cord blood samples of neonates studied in the United Arab Emirates, with mean levels of 1040 pg/ml and 1880 pg/ml, respectively (274).

Of 282 cord blood samples from Ghana, 101 samples from Kenya, and 78 samples from Nigeria, aflatoxins were detected in 31%, 37%, and 12%, respectively (275, 276). Though the detection rate of AF-alb in maternal blood samples was not stated, the levels of AF-alb found in 755 Ghanaian mothers in a cross-sectional study were reported to range from 0.44 to 268.73 pg/mg (29). Detectable levels of aflatoxins were found in 22% to 82% of cord blood of Nigerian neonates (277, 278), and 58% of the cord blood samples from Sierra Leone (279). AF-alb was detected in 29 of 30 (97%) maternal blood samples, and 22 of 30 (70%) matched umbilical cord blood sera from Gambian neonates (280). Turner et al. (2007) found AF-alb ranging from 5 pg/mg to 896 pg/mg in 48 out of 99 (48%) Gambian cord blood samples.

The studies in Kenya (275) and Sierra Leone (279) showed higher detection rates of aflatoxins in maternal blood samples (53% and 75%) compared with cord blood (37% and 58%). By contrast, aflatoxins (AFB_1, AFG_1, AFQ_1) were detected with much greater frequency in Thai

cord blood samples (17 of 35) compared with the maternal blood samples (2 of 35), indicating trans-placental transfer of aflatoxins from mothers to fetuses (281). However, low trans-placental transfer of AF-alb or low efficiency of fetal metabolism to change free aflatoxins to the AF-alb form has been suggested, because of the much greater levels (up to 10 times) of AF-alb in the venous blood of Gambian mothers compared with those in matched cord blood samples (280).

One study conducted to determine the efficacy of fetal-specific CYP3A7 and adult specific CYP3A4 in hamster rat found similar level of enzyme expressions to activate AFB_1 in both CYP3A lines (282). An *in vitro* study was conducted in guinea pigs to compare the formation rates of aflatoxin-DNA adduct and AF-alb between adult and second trimester prenatal livers. Whereas lower expression of two aflatoxin detoxification enzymes, microsomal epoxide hydrolase and polymorphic glutathione S-transferase, and higher expression of lipooxygenase – an enzyme which can activate AFB_1 to form AFB_1-DNA adduct (283) – were detected in prenatal livers compared with adult livers, the formation rates of DNA-adducts and protein adducts in prenatal livers and adult livers were not different (284). Recently, a human *in vitro* study showed that AFB_1 was metabolized by human placentas to aflatoxicol, a less mutagenic but equally carcinogenic metabolite of AFB_1 (285). However, fetal metabolism can be greatly different from adult metabolism as a result of reduced hepatic blood flow and incomplete hepatic formation. Still, little is known about biotransformation of aflatoxins in fetuses, and further studies are needed.

The presence of aflatoxins, particularly AFM_1, in maternal breast milk in several regions indicates that children worldwide may be exposed to aflatoxins through breastfeeding. Though AFM_1 was found in none of the breast milk samples from French (286) and German mothers (287), about 30% to 60% of breast milk samples from Sudanese (288), Kenyan (275), Ghanaian (275, 276), and Egyptian mothers (289, 290) contained detectable levels of aflatoxins. In Sierra Leone, 99 of 113, or 88%, of breast milk samples from mothers contained detectable levels of aflatoxins (291). However, only 11% of the breast milk samples from Zimbabwean mothers (286) and 5% of breast milk samples from mothers in Cameroon (292) were AFM_1 positive.

In Asia and the Middle East, AFM_1 was detected in 20 out of 91 breast milk samples of Iranian mothers with the mean concentration of 6.96±0.94 pg/ml (293). About 45% of Thai mothers had detectable AFM_1 in breast milk with a median concentration of 664 pg/ml. The AFM_1 levels in Thai mothers ranged from 39 pg/ml to 1736 pg/ml (294). Very high percentages of the UAE mothers– more than 90%– had detectable levels of AFM_1 in the breast milk (30,

295). Whereas two prior studies detected aflatoxins in only one of 231 (0.4%) and eight of 61 (13%) breast milk samples from Turkish mothers (296, 297), aflatoxins were detected in all of 75 breast milk samples from the lactating Turkish mothers in a more recent study (298). This discrepancy may have many causes, including differences in analytical methods, differences in study populations, or issues of seasonality.

Gong et al. (2003, 2004) found that in Benin and Togo childhoods, AF-alb levels increased with age until three years old. This trend reflected the transitioning of children from breastfeeding to weaning and post-weaning foods. Children who were completely weaned had higher levels of AF-alb than breastfed or partially breastfed children.

Because of multiple routes of exposure beginning from the fetal environment, high percentages of children in various countries have been exposed to aflatoxins, as detected in multiple studies. About 85% to 100% of children in African countries, such as Gambia, Guinea, Kenya, Benin, Togo and Senegal had either detectable levels of serum AF-alb or urinary aflatoxins (22, 36, 272, 299-302, 303, 1993). Levels of AF-alb in children from industrial nations are typically significantly lower than those living in less developed countries. Wild et al. (1990) found AF-alb levels as high as 350 pg/mg in almost all sera of children in various African countries. By contrast, none of the French or Polish sera contained AF-alb at levels higher than 5 pg/mg albumin (303).

The seasonality of sampling has been addressed in a number of studies (273, 275, 276, 278, 290, 300, 303, 304). Many studies had detected aflatoxins in human body fluids more often in the wet season than the dry season. Some of them include the studies which determined aflatoxins in the cord blood samples from Kenya and Nigeria neonates (275, 278), AFM_1 in the breast milk of Ghanaian mothers (276), and aflatoxins in the Taiwanese breast milk samples (273). Similarly, AFB_1, AFB_2, AFG_1, and aflatoxicol were detected more often in the urine samples of Sierra Leone children collected during the rainy season, compared with the dry season (304). On the other hand, in two Gambian studies, AF-alb was detected more frequently in the serum of Gambian children collected during the dry season than the wet season (300, 305). Aflatoxin development in storage, after groundnuts had been harvested at the end of the wet season, was expected to be the cause of the elevated levels of AF-alb in the dry season (300, 306).

5.4.2 Past studies on aflatoxin and kwashiorkor

An area of inquiry that had gained notice several decades ago concerned the possible link between aflatoxin exposure and childhood kwashiorkor, a disease of protein energy malnutrition. Both kwashiorkor and marasmus (another childhood condition common in less developed countries) are diseases of severe malnutrition. While protein deficiency is a major etiology of either kwashiorkor or marasmus, one key difference between these two conditions is that kwashiorkor can occur even when caloric intake of the children is sufficient, while marasmus can be caused by deficient caloric intake. Children with marasmus are less likely to suffer from fatty liver or edema: classical manifestations of kwashiorkor. Other symptoms of kwashiorkor include light-pigmented hair and skin and anorexia (307). An individual with edema from kwashiorkor and wasting from marasmus is considered to have marasmic kwashiorkor (308). While marasmus is sometimes perceived as an adaptive response to starvation, kwashiorkor is considered a maladaptive response to undernourishment (307, 309, 310).

Since the 1980s, several studies have examined the possible association between aflatoxin exposure and kwashiorkor (292, 311-316). These studies found that aflatoxins or their metabolites were detected with greater frequency in the blood or urine of children with kwashiorkor than in healthy children or children with other protein malnutrition-related conditions, such as marasmus. Moreover, aflatoxins were detected more frequently, but not statistically significant, in autopsies of lungs and livers, but not in kidneys, of children who died from kwashiorkor; compared with those who died from other diseases or other forms of malnutrition (312, 315, 317). It is worthwhile to note that only seven liver specimens were included in Lamplugh and Hendrickse, 1982−three from kwashiorkors, three from marasmic-kwashiorkors and one from marasmic children.

Other factors could explain these phenomena, however. In a study conducted in a hospital in Durban, South Africa (318), children with kwashiorkor were matched with controls with no symptoms of protein energy malnutrition. Aflatoxins were detected in the serum and/or urine of all children. The serum/urine ratio was significantly higher in the kwashiorkor group; the controls, however, had a higher proportion of urine aflatoxins than the kwashiorkor group. These findings may reflect impaired liver function in kwashiorkor, which could in turn lead to differences in how aflatoxin is metabolized; rather than aflatoxin's playing any direct role in

causing kwashiorkor. Indeed, it has been proposed that children who suffer from kwashiorkor are at greater risk to the hazards of dietary aflatoxin (37).

5.4.3 Aflatoxin and growth impairment in children

Various studies have demonstrated that aflatoxin exposure, through a variety of sources as described above (*in utero*, through maternal breast milk, and in weaning diets), is linked with growth impairment. Table 5-4 summarizes the studies that have shown an association between aflatoxin exposure and various measures of growth impairment in human children.

Table 5-4. Human studies of the effects of aflatoxin on growth impairment.

Country/ Study population	Results	Study
Benin/ Children ages 16–37 months (n=200)	a) Significant negative correlation ($p < 0.0001$) between AF-alb adduct and height increase over 8-month study period b) A mean 1.7 cm reduction difference in growth over 8 months in the AF-alb adduct highest quartile, compared with the lowest quartile c) No association between AF-alb and micronutrient levels	Gong et al. (2004)
Benin and Togo/ Children ages nine months to five years (n = 480)	a) Negative correlation between individual AF-alb adduct and HAZ, WAZ, WHZ ($p=0.001$, 0.005, 0.047 respectively) b) Factors influence AF-alb adduct level: age (up to 3 years) and weaning status ($p= 0.0001$) c) Twofold higher mean AF-alb adduct levels in weaned children than those receiving a mixture of breast milk and solid foods after adjustment for age, sex, agro-ecological zone, and socioeconomic status	Gong et al. (2002, 2003)
The Gambia/ Children ages six to nine years old (n= 472)	a) 93% AF-alb adduct detection rate b) Geometric mean level = 22.3 pg/mg; range 5–456 pg/mg) c) Significant association between AF-alb adduct and the weight for height score ($p=0.034$) d) Significant lower levels of sIgA in children with detectable AF-alb adduct compared with those with non-detectable levels [50.4 µg/mg protein (95% CI: 48.0, 52.8) and 70.2 µg/mg protein (95% CI: 61.1, 79.2) respectively, $p<0.0001$]	Turner et al. (2003)
The Gambia/ Infants to one year old (n=138)	a) The geometric mean AF-alb adduct levels = 40.4 pg/mg (4.8–260.8), 10.1 pg/mg (5.0–189.6), and 8.7 pg/mg (5.0–30.2) in maternal, cord and infant blood, respectively b) Associations between the reduction of maternal AF-alb from 110 pg/mg to 10 pg/mg & 0.8 kg increase in **weight** & 2 cm increase in **height** of children within first year of life c) AF-alb adduct in maternal blood as a strong predictor of both **weight** ($p = 0.012$) and **height** ($p = 0.044$) gain, with lower gain in those with higher exposure	Turner et al., (2007)

Table 5-4 (continued)

Country/ Study population	Results	Study
Ghana/ Pregnant women and their infants (n=785)	a) Odds ratio of having low birth weight babies in the highest AF-alb adduct quartile mothers is 2.09 (p=0.007)	Shuaib et al., (2010)
Iran/ Lactating mothers (n= 160: 7 preterm delivery mothers, and 153 with full term infants)	b) 98% (157 of 160 breast milk samples) AFM_1 positive detection rate (average concentration: 8.2±5.1 ng/kg, range: 0.3–26.7 ng/kg) c) Significant association between aflatoxin and height at birth of infants ($p<0.01$)	Sadeghi et al. (2009)
Iran/ Lactating women (n=182: 91 from urban areas in Tabriz and 91 from rural area)	a) 22% and 0% detection rates of AFM_1 in breast milk of mothers living in rural areas, and urban areas, respectively b) AFM_1 : 6.96±0.94 pg/ml c) Significant association between the HAZ of infants and maternal AFM_1 ($p<0.015$) d) Significant lower scores in HAZ and WAZ of infants born to AFM_1–positive mothers ($p<0.05$)	Mahdavi et al. (2010)
Kenya/ Children ages 3–36 months (n =242)	a) Significant association between numbers of children who were wasting and were being fed on flour contaminated with aflatoxin ($p=0.002$)	Okoth and Ohingo (2004)
United Arab Emirates/ Pair maternal -cord blood samples from women admitted 1995–1998 (n=166 pairs)	a) 100% cord blood AFM_1detection rate in low birth weight neonates VS 55% detection rate in normal weight neonates b) Strong negative correlation between aflatoxin levels and birth weights ($r =–0.565$, $p <0.001$)	Abdulrazzaq et al. (2004)

Note: AF-alb: aflatoxin albumin adduct, HAZ, height for age z-score, WAZ, weight for age z-score, WHZ: weight for height z-score.

The studies that examined these associations were conducted primarily in the Middle East and in Africa. In a study in the United Arab Emirates, Abdulrazzaq et al. (2004) detected AFM_1 in 100% (43 of 43) of neonates born with low birth weights, but only in 55% (68 of 123) of

neonates with normal birth weight. Aflatoxin levels in the cord blood and maternal blood samples were inversely associated with weight at birth ($r = -0.565$, $p = 0.001$ and $r = -0.654$, $p=0.0001$) (274).

Two recent Iranian studies have linked AFM_1 levels in mothers' breast milk with growth impairment in babies. AFM_1 was found in 157 of 160 (98%) of breast milk samples collected from Iranian mothers living in Tehran, with concentrations ranging from 0.3–26.7 ng/kg. The levels of AFM_1 in breast milk were inversely correlated with length of infants at birth ($p<0.01$) (31). Another study collected breast milk from mothers living in urban areas of Tabriz and its surrounding rural areas. Only 22% of breast milk samples from mothers in the rural surroundings of Tabriz contained detectable levels of AFM_1, and none of the breast milk samples from mothers living in urban areas of Tabriz were found to have AFM_1. The levels of AFM_1 in the breast milk of mothers from rural areas of Tabriz ranged from 5.1–8.1 pg/ml. There was a significant inverse relationship between AFM_1 levels in maternal breast milk and the height-for-age z-scores (HAZ) in infants 90–120 days old ($\beta=-0.31$, $p<0.015$). The children whose mothers were AFM_1-positive had lower HAZ and weight-for-age z-scores (WAZ) than children born to mothers with no detectable AFM_1 (293).

In Africa, studies associating aflatoxin exposure with growth impairment in children were conducted in Kenya and several West African nations. In an early study on 125 babies in rural Kenya (319), aflatoxins were detected in 53% of mothers' blood, and the mean birth weight of females born to mothers whose blood tested positive for aflatoxin was significantly lower (255 g) than those born to mothers with no aflatoxin detected in the blood. Additionally, the two recorded stillbirths were both to mothers who tested positive for aflatoxins.

A series of studies conducted in Togo and Benin in the early 2000s provides insightful information into the cross-sectional, longitudinal, and dose-response aspects of the association between aflatoxin exposure and childhood growth impairment (35, 36, 272). In a cohort of 480 children aged 9 months to 5 years in these two countries, the prevalence of stunting and underweight were reported to be 33% and 29%, respectively (35, 272). AF-alb was detected in 99% of the children, with a geometric mean level of 32.8 pg/mg (95% CI: 25.3, 42.5). Clear dose-response relationships were found between mean AF-alb levels and lower HAZ and WAZ scores. Children who were stunted ($HAZ \leq -2$) had 30–40% higher mean AF-alb levels compared with non-stunted children. Household socioeconomic status and maternal education

were not significantly associated with AF-alb levels in children. There was no consistent pattern between the socioeconomic status of the mothers and the adduct levels in the children (35, 272). A subsequent eight-month longitudinal study in 200 children aged between 16 and 37 months in Benin showed a significant negative association between height velocity, but not weight, and mean AF-alb levels (36). A difference of 1.7 cm over the eight-month study period in adjusted height between the highest and lowest AF-alb quartile was observed.

Unlike Gong et al. (2002), a study in Gambia on a cohort of 472 children between 6–9 years old did not find that AF-alb levels were associated with HAZ or WAZ (22). It is noteworthy that the participants in the Gambian study were born during the implementation of a five-year maternal supplementation program, in which pregnant mothers were given two groundnut biscuits daily which provided 4,250 kJ, 22 g protein, 56 g fat, 47 mg calcium, and 1.8 mg iron per day to the mothers (320). However, a subsequent study did find an association between *in utero* aflatoxin exposure and growth impairment. Following 138 Gambian neonates for one year, Turner et al. (2007) found a significant association between aflatoxin exposure in mothers during pregnancy and height and weight gain of their infants in the first year of life. They concluded that if the maternal AF-alb levels dropped from 110 pg/mg to 10 pg/mg, the weights and heights of one-year old infants would increase by 0.8 kg and 2 cm on average, respectively (32).

In a study in the Kisumu District of Kenya (321), weaning flours from 242 households with children aged 3–36 months old were analyzed for aflatoxins. The weights and heights of the children were measured to determine prevalence of stunting, underweight, and wasting. While only 28% of non-wasted children were from households with aflatoxin contaminated flour, about 54% of the wasted babies were from households with detectable aflatoxin in the flour. There was a significant association between aflatoxin exposure and wasting ($p=0.002$). Aflatoxins were also more frequently detected in the flour of stunted and underweight children compared with normal children. However, these differences were not statistically significant (321).

In a recent cross-sectional study, Shuaib et al. (2010) found levels of AF-alb ranging from 0.44 to 268.73 pg/mg in maternal blood samples from 755 Ghanaian mothers. After adjusting for sociodemographic variables, it was found that the mothers in the highest quartile of AF-alb levels were at significantly higher risk of having babies with low birth weight, defined as being below 2.5 kg (OR=2.09). There were also increased odds of having preterm deliveries and

stillbirths with for mothers who had AF-alb in the highest quartile, though the associations between AF-alb and these risk factors were not statistically significant (29).

5.4.4 Childhood weaning foods

A focus of interventions to reduce aflatoxin exposure in childhood could be on improving the quality and composition of weaning foods. In Africa and Latin America, childhood weaning foods are usually prepared from maize (322), which can lead to high aflatoxin exposures early in life. Several maize-based foods such as gruel, ogi (fermented maize gruel), pap (maize porridge), and eko – boiled and gelatinized ogi (323) – are used as weaning foods in Africa. Groundnuts can also be commonly used as a weaning food in various African regions.

The weaning process in the West African countries starts in many cases at early ages, when the children are about 3–6 months old (324). Up to 50% of children in Makurdi, Nigeria, consume pap as their main weaning food, followed by Cerelac, a commercial infant formula (26.5%) and pap mixed with other food (11%) (325). Weaning foods in West Africa are usually made of maize, groundnuts, sorghum, millet, and guinea corn (28). Likewise, maize is a major weaning food in some countries in East Africa. In Uganda, 89% of children are fed maize porridge regularly. About 24.5% of children aged three to 28 months have maize porridge seven days a week (326). Gruels prepared from maize are used as weaning foods in Kenya (327), Tanzania (324), and Ethiopia (328). Other staple crops are also used to prepare weaning foods in these East African countries. Some of them include sorghum in Tanzania, sorghum and millet in Kenya, barley and wheat in Ethiopia. Sorghum porridge (nasha) is a traditional weaning food in Sudan (329).

Many children in Latin America also consume large amounts of maize in their weaning diets, which can increase aflatoxin exposure. Maize-based gruels are among several kinds of Brazilian weaning foods, which also include rice flour or cassava flour-based gruels, cassava, sugar, and diluted milk (330). Maize tortillas consumed with milk, beans, bread, pasta, fruit, chicken soup, flavored gelatin, or soft drinks are commonly used as weaning foods in Mexico (331).

Children's weaning foods in Asia vary substantially from region to region. Weaning foods in China are whole eggs, vegetables and fruits, porridge (rice, maize, or wheat), and infant formula (332-335). Maize and even rice are contaminated with aflatoxin in many parts of China (336-340). In 1992, maize consumption among residents of Guangxi, where HCC prevalence is among the highest in the world, was as high as 350–500 g/day (337). In India, children may be weaned on various kinds of food: formula, porridges (maize, rice, millet, etc), commercial cereals, pulses, fruit, rice with milk and/or ghee, roti, and potatoes (341-343). Nepalese children are weaned on porridge and animal milk at ages of 2.5–6.5 months old. Thai weaning foods include rice-based food, fruit juice, fruit, meat, fish and vegetable soup (318, 344).

Because aflatoxin exposure in children increases markedly following weaning (272) and is associated with multiple adverse health outcomes, reducing aflatoxin levels in weaning foods is crucial in high-risk regions of the world. Interventions could include dedicating cleaner maize and groundnuts to weaning foods, or provision of weaning foods that contain wide varieties of food crops instead of few food crops.

5.5 DISCUSSION

Growth impairment in children is a pervasive public health probl in low- and middle-income countries worldwide, and is associated with a wide variety of factors such as poor nutrition, poor hygiene, socioeconomic status, local political instability, repeated infectious diseases, and environmental toxins (229). Aside from adverse health effects associated with childhood growth impairment, such as cognitive impairment and increased risk of infectious diseases and death, there are also economic consequences: childhood undernutrition as indicated by stunting has been associated with lower human capital in low- and middle-income countries (345). Stunting, wasting or underweight is associated with increased mortality risks in childhood (229) Reducing risk factors for growth impairment in children age under five could be a way: One of the eight goals to improve socioeconomic and human health, endorsed by the leading international organizations and world leaders in 2000, is to reduce mortality rate among children age under

five, by two-thirds in 2015 (346). Reducing risk factors for growth impairment in children age under five can be another way, along with other interventions, to achieve this goal.

Among the risk factors associated with growth impairment, aflatoxin emerges as playing a potentially important contributory role. The weight of evidence linking aflatoxin with growth impairment has been increasing over the last five decades of research: first primarily in animal studies, and in the last decade increasingly in epidemiological studies. When considering the Bradford Hill criteria for causality (347), the recent epidemiological studies have provided useful supporting evidence. When controlling for other socioeconomic and environmental factors, the strength of association between aflatoxin and stunting and underweight is strong. Moreover, the dose-response relationship between aflatoxin exposure and growth impairment is monotonically increasing (35), which is consistent with a causal effect, although other confounding factors cannot be excluded (148). Animal and epidemiological studies are concordant in their findings.

One critical piece of information that is currently unavailable is a mechanism by which aflatoxin causes growth impairment in humans and animals. If such a mechanism could be elucidated, then the weight of evidence linking aflatoxin with growth impairment would become even stronger. Though this exact mechanism has yet to be identified, several have been proposed. One is that immunomodulation associated with aflatoxin exposure (22, 348) can cause recurrent infections in children, which can lead to growth impairment (27). Another is that changes in intestinal integrity (possibly in part resulting from immunomodulation) could make hosts more vulnerable to intestinal foreign microbes (27). Other possible mechanisms include down-regulation of genes associated with energy production and fatty acid metabolism (349), impairment of protein synthesis and the inability to mobilize fat (241), and changes in hepatic metabolism of vitamins and micronutrients (350).

Given the increasingly strong evidence that aflatoxin contributes to growth impairment in children, and the knowledge that it is a common contaminant of weaning foods in many parts of the world where childhood stunting is prevalent (e.g., sub-Saharan Africa and Asia), it is important to attempt to reduce aflatoxin exposure in foods consumed by children. Multiple aflatoxin control strategies have been developed to lessen aflatoxin exposure by reducing aflatoxin development in fields, during storage or reducing aflatoxin bioavailability. We previously reviewed the cost and efficacy of various types of aflatoxin control methods (150), and reported that at least two aflatoxin control interventions were cost-effective to reduce

aflatoxin in maize in Nigeria and groundnuts in Guinea, respectively (135). However, implementing aflatoxin control interventions needs extensive involvement from multiple stakeholders, from the levels of individuals to national and international institutions. Moreover, in the parts of the world where they are most needed, aflatoxin risk-reduction interventions must be evaluated for feasibility: safety, standardizability, characteristics of delivery, requirements on government capacity, and usage characteristics, among other factors (351).

In summary, aflatoxin appears to play a contributory role in growth impairment in both children and animals. In children, aflatoxin exposure is especially problematic in parts of the world where maize and groundnuts are dietary staples. Childhood exposure to aflatoxin can occur *in utero*, in mothers' breastmilk, and particularly in weaning foods. Aflatoxin-associated growth impairment can, in turn, contribute to increased risk of mortality and morbidity in children worldwide. Strategies should focus on reducing aflatoxin exposure in children and mothers' diets, in ways that are cost-effective and technically feasible in parts of the world where aflatoxin risks are especially high.

6.0 AFLATOXIN AND GROWTH IMPAIRMENT IN NIGERIA

6.1 ABSTRACT

Aflatoxin not only induces hepatic cancer, but may also impair child growth development. A growing body of evidence from animal and human studies indicated the negative impacts of aflatoxin exposure on growth performance. Stunting, a condition defined as a child's height-for-age score below minus two standard deviations from the mean standard height, is highly prevalent in Nigeria. We performed this study to supplement our prior investigation on t he analysis of the costs of a particular aflatoxin control intervention to prevent one healthy life year lost due to liver cancer. We previously found that the HBV vaccine provided a significant lower cost to avert one disability life year compared with the aflatoxin control interventions. In this study, we included the benefits from preventing stunting to the outcome of three interventions: bicontrol, the postharvest intervention package, and NovaSil. Our results showed that dietary aflatoxin exposure would cause about 0–18% of stunting in children aged five years and below, depending on aflatoxin levels in food crops. At the upper bound level of aflatoxin, biocontrol and NovaSil clay would reduce aflatoxin exposure to levels that cannot cause stunting; the postharvest intervention package would prevent 5% of children from becoming stunted. Each single intervention is cost-effective to prevent stunting in Nigeria.

6.2 INTRODUCTION

In the previous chapter, we have reviewed a number of studies showing the associations between aflatoxin and growth impairment. In several animal species, including chickens, pigs, rats, mice, and fish, aflatoxin has shown to reduce average daily feed intake (ADFI) , reduce weight gain and increase feed conversion ratios (FCR)−the ratios between amounts of feed intake and weight gain. Furthermore, reduced birth weights following *in utero* exposure to aflatoxin were presented in both animal and human studies. The epidemiological studies from several countries, such as Benin, Togo, Gambia, Ghana, Iran, Kenya, and UAE, focusing on the associations between aflatoxin and growth faltering have been released during the past decade. These studies showed significant negative associations between aflatoxin exposures and growth performances in children.

Though there are a number of growth performance parameters, such as weight-for-age, height-for-age, and weight-for-height, the parameters usually used to indicate chronic malnutrition is the impairment in height-for-age. Children whose height-for-age scores (HAZ) are below two standard deviation (SD) of the mean height-for-age standard is classified as stunted.

Recently, there was a study showing that children who were stunted were more likely to die from various diseases. This study, following children from birth to the age of three years in 36 countries, found that children, whose height for age scores (HAZ) were between two and three standard deviation (SD) below the means HAZ, were 1.6 times more likely to die from diarrhea, malaria, and respiratory tract infections compared with children whose HAZ were higher than –1SD HAZ score. The mortality rate from diarrhea, pneumonia, malaria, and measles in children who were severely stunted (HAZ< –3SD) were increased to four times higher than children whose HAZ >–1SD (229).

To the best of our knowledge there is no study conducted in Nigeria to determine burdens of growth impairment related to aflatoxin. Based on the WHO database about 43% of Nigerian children age five years old and below were estimated to be stunted (233). Currently, the number of children age 0–5 years old in Nigeria is about 23.6 millions (196), which means about 10.1 million Nigerian children are being stunted.

6.2.1 Odds ratio between aflatoxin and growth impairment in humans

A number of human studies have determined the relationships between aflatoxin and growth performances. But they differ in either methodologies or study populations. For example, a study conducted in Kenya determined the associations between the presence of aflatoxin in family flour and growth performances in children aged 3-36 months; whereas, a UAE study exhibited the relationships between *in utero* aflatoxin exposure and birth weights. A number of studies conducted in Benin, Togo, and Gambia used AF-alb adduct as an dietary aflatoxin biomarker and determined the associations between AF-alb adducts levels and various growth performance parameters, such as height-for-age, weight-for-age, and height-for-weight. Some of these studies provided enough data to determine odds ratios, a measure of the strength of an association.

Table 6-1 lists three studies and the odds ratios between aflatoxin exposure and growth performance parameters. Between October 2000 and March 2002, Okoth and Ohingo collected weaning flour samples from 242 households from Kisumu District, Kenya, and assessed growth performances of children aged between 3-36 months in these households (321). Prevalence rates of being stunted, underweight, and wasted in the studied populations were: 34%, 30%, and 6%, respectively. Compared with the normal children, a significant higher percentage ($p=0.002$) of the wasted children was fed on aflatoxin-contaminated flour (53.8% and 27.7%). About 28.9% of the normal children and 32.4% of the stunted children were fed aflatoxin contaminated flour ($p=0.67$). There was a non-significant difference between the percentages of normal children and underweight children who were fed the contaminated flour (27.3% and 41.4%, $p= 0.13$). Based on the Wellcome Classification of Severe Protein Energy Malnutrition, 66% of malnourished children had aflatoxin contaminated flour, while only 27.4% of nourished children were fed aflatoxin contaminated flour ($p=0.004$).

The associations between AFM_1, an aflatoxin metabolite, in newborns and their mothers and low birth weight were studied by Abdulrazzaq et al. 2004 (274). They collected 250 cord blood and maternal blood samples from 1,500 women admitted to the labor wards in two UAE hospitals between 1995 and 1998. A ll of the low birth weight neonates ($n=43$) were AFM_1 positive (> 10 mg/ml); whereas, only 68 of 123 normal weight neonates had AFM_1 higher than

10 mg/ml in their cord blood. The correlation coefficient between AFM_1 levels in cord blood and birthweight was –0.565 (p=0.001).

In order to determine the odds ratio when none in the low birth weight groups was AFM_1-free, we added 0.5 to all four cells in 2x2 table to treat the problem of having a zero value in the table (352, 353). The odds of being low birth weight in a baby, whose cord blood contains AFM_1, was 70 times higher than a baby, whose cord blood was free from AFM_1.

Instead of measuring aflatoxin in food, Gong et al. 2002 determined peripheral blood AF-alb adducts levels of 480 Benin and Togo children aged between nine months to five years. About 99% of children contained detectable levels of AF-alb adducts with a geometric mean of 32.8 pg/mg albumin. Among 475 children who had detectable levels of AF-alb adducts, 148 children and 129 children were stunted and underweight, respectively (35).

Regarding WHO definition of stunting, as long as the HAZ are more than the negative two standard deviation of WHO growth standard, the children are not classified to be stunted, even if aflatoxin deteriorates growth performance in children. In order to obtain the odds ratio between AF-alb adducts and growth performances from Gong et al, 2002, we classified children into quartiles based on their AF-alb adduct levels and compared the odds of being stunted and underweight between the children in the lowest quartile and the children in the upper three quartiles. Each quartile contained 113–114 children. Of total 479 children, 307 children, whose serum AF-alb adducts levels were less than 32 pg/mg albumin, were not stunted, and 148 children, who contained higher AF-alb adducts levels, were stunted. The AF-alb adducts levels and HAZ of 24 children were not mentioned. We assumed that their HAZ and AF-alb adduct levels did not follow the dose-response relationship. We divided these 24 children equally into quartiles and put six and 18 into the lowest quartile and three upper quartiles, respectively to obtain the highest possibility that these 24 children did not follow the dose-response relationship. Therefore, the three upper quartiles consisted of 114 non-stunted and 148 stunted children and the lowest quartile contained 211 non-stunted children and six stunted children. The odds of children whose AF-alb adduct levels were in the three upper quartiles having been stunted was about 13 times higher than the lowest quartile children.

Similarly, the odds ratio of underweight children to having had high AF-alb levels is about 9.05. Of 480 children, 113 and 230 normal weight children had AF-alb adducts in the first quartile and the three upper quartiles, respectively. Only seven underweight children contained

low levels of AF-alb adduct (first quartile) compared with 129 underweight children, whose AF-alb adduct levels were in the three upper quartiles. The odds ratio suggests that children, whose AF-alb adducts levels were in the upper three quartiles to be stunted, and underweighted, were 13 times, and 9 times higher than children whose AF-alb adducts levels were low.

Table 6-1. Odds ratio between aflatoxin and growth performance

Countries	Subject	Aflatoxin	Growth parameter	Odds ratio
Benin and Togo (35)	Children aged <5 years old	AF-alb adduct (the three upper quartiles versus the lowest quartile)	Stunting	13.32
			Underweight	9.05
UAE (274)	New born babies	Cord blood AFM_1	Low birth weight	70
Kenya (321)	Children 3−36 months	Aflatoxin in family flour	Malnourished*	3.95
			Wasted	3.16
			Stunted growth	1.20
			Underweight	1.86

Note: [a]Significant association between the HAZ of infants and maternal AFM_1 ($p<0.015$), Significant lower scores in HAZ and WAZ of infants born from AFM_1 –positive mothers ($p<0.05$).

Though the odds ratio can identify the strength of the association between aflatoxin and growth parameters, it does not provide much information on the burdens of growth impairment in a particular area. In this study, we employed data from Gong et al. 2002 to develop dose

response functions between AF-alb adducts levels and proportions of stunting and adopted these functions to determine the burdens of stunting in Nigeria.

6.3 METHODS

6.3.1 Dose-response relationships development

The dose-response relationships between AF-alb levels and HAZ scores, along with the numbers of children in each HAZ score group in Gong et al. 2000 (35), were transformed to the dose-response functions between AF-alb and proportions of stunting. The children whose HAZ were below -2SD of HAZ were considered to be stunted. Then we identified the cumulative numbers of stunted and non-stunted children in each adduct level for HAZ groups. Twenty-four children, whose HAZ were not mentioned in Gong et al. 2002, were classified to be either stunted or non-stunted. We generated two linear regression functions based on the transformed data. The lower and upper bound functions were obtained when the 24 children were considered to be non-stunted and stunted, respectively.

6.3.2 Burdens of aflatoxin-related stunting in Nigeria estimation

We estimated AF-alb adduct levels in Nigeria based on the Nigerian aflatoxin dietary exposure levels calculated in the previous chapter, using the conversion factor from Shephard 2008 (37). Later, we determined the estimated numbers of aflatoxin-related stunting cases, and the numbers of stunting cases prevented if biocontrol, the postharvest intervention, and NovaSil clay were adopted in Nigeria using the two aforementioned dose-response functions.

6.3.3 Cost-effectiveness assessment

Though in general childhood growth performances refer to the growth performances in children age 0-5 years old, some studies found that stunting prevalence peaked and reached the plateau when the children were aged about three years old. The numbers of prevented DALYs were discounted by 3% per year by three years to account for future benefits from current investment. The efficacy of the interventions consisted of two parts, efficacy in preventing liver cancer and efficacy in preventing aflatoxin-induced stunting. The methods to calculate the costs of aflatoxin control interventions and the efficacy in preventing liver cancer were already presented in chapter 5. The numbers of stunted cases were multiplied by stunting DALY per case, assuming 5% of cases would die because of stunting-related conditions. The numbers of DALY per survival case (0.23 DALY per case), and dead case (33.2 per case) obtained from Bhutta et al. 2008 (354).

6.3.3.1 Cost-effectiveness ratio (CER)

The CER– the ratio between the cost and the benefit of a particular intervention to determine the cost to prevent one healthy life year lost–of each intervention was compared with the Nigerian GDP per capita, which was about $2,360 in 2010 (211). Based on this ratio, each intervention was assigned to one of three following categories proposed by WHO (130): highly cost-effective (CER< one GDP per capita), cost-effective (one GDP<CER<three GDP), and not cost-effective (CER> three GDP per capita).

6.4 RESULTS

6.4.1 Dose –response relationship

Table 6-2 shows the numbers of children with the average AF-alb adduct levels of 17.0, 26.4, 27.9 and 29.1 pg/mg albumin based on Gong et al. 2002 (35). In that study, they determined the adduct levels in 480 Benin and Togo children aged between nine months and five years. One sample was lost, and 4 samples were free of AF-alb adducts, leaving 475 samples in their analysis. But, only 455 samples were presented in dose-response relationship between AF-alb adduct levels and the HAZ. We included 24 children (four AF-alb free and 20 non-mentioned children) in either the non-stunting group (the lowest possible stunting proportion) or the stunting group (the highest possible stunting proportion).

Table 6-2. Prevalence of stunting and aflatoxin albumin adducts

Mean adduct levels	Numbers of children	Lowest possible stunting proportion	Highest possible stunting proportion
17.0	25	0%	7.25%
26.4	282	0%	7.25%
27.9	108	24.6%	30.07%
29.1	40	30.9%	35.91%

Note: (adapted from Gong et al., (2002))

The linear regression functions between the levels of AF-alb adduct and proportions of stunting were:

$$Y = 0.0196X - 0.2918$$

Equation 6: Levels of AF-alb and proportions of stunting (lower bound)

and

$$Y = 0.0212X - 0.3928$$

Equation 7: Levels of AF-alb and proportions of stunting (upper bound)

Where X= AF-alb adduct levels, and Y = proportions of stunting

To the best of our knowledge, aside from Gong et al. 2002, t here was only one study providing data of AF-alb adduct levels and proportions of stunting, Turner et al. (2003). Turner et al. (2003) determined the geometric means of AF-alb adduct levels of 472 Gambian children aged six years to nine years old to be 22.3 pg/mg albumin. The calculated stunting prevalence rates in Gambia based on our dose-response functions were 8.0% and 14.5%. The average from the upper and lower calculated stunting prevalence was 11.3%. (actual prevalence = 11.5%)

6.4.2 Potential aflatoxin-related stunting in Nigeria

In general, children consume three to four times much more food per kilogram body weight than adults (355). Moreover, several food taboos arose in Africa can hinder children from having varieties of food. Therefore, children are more likely to be exposed to aflatoxin compared with adults. To convert adult dietary aflatoxin exposure to aflatoxin exposure in children, we multiplied maize and groundnuts consumption rates adopted from FAOSTAT by five. Table 6-3 shows the estimated stunting prevalence as the results of maize, groundnut, and both maize and groundnut consumptions.

The results presented in table 6-3 accentuate the need of employing total aflatoxin exposure levels in the analysis of aflatoxin-induced stunting. Aflatoxin levels in groundnuts, without any aflatoxins from other sources, cannot produce stunting. However, when combined with aflatoxin exposure in maize, aflatoxin in groundnuts adds 7.4% more stunting proportions than that produced by aflatoxin in maize alone. Consuming groundnuts increases levels of total aflatoxin exposure children receiving per day. Our results are in good agreement with the data from Gong and colleagues in 2003 that groundnuts consumptions do not correlate with AF-alb adduct levels in Benin and Togo children aged less than five years; however, they expressed their awareness of an importance of aflatoxin in groundnuts on the levels of total aflatoxin exposure (272).

Table 6-3. Potential stunting burden in Nigeria from consuming maize and groundnuts contaminated with aflatoxins

Items	Staple crops		
Staple crops	Maize	Groundnuts	Total
Average aflatoxin levels (ng/g)	13.4−36.2	64.8−67.5	−
Children consumption rate (g/kgBW-day)	6.14	0.54	−
Aflatoxin exposure (ng/kgBW-day)	82.0−222.15	35.0−36.46	117.0−258.61
Estimated AF-alb adducts from chronic ingestion (pg/mg albumin)[a]	8.2−22.22	3.5−3.65	11.7−25.87
Percent of stunted children[b]	0−11.1	0	0−18.5
Expected no. of stunted children	0 to 2,619,600	0	0 to 4,377,800

Note:[a]a 100 pg AF-alb adduct/mg albumin is expected to be a result of chronic ingestion of 1000 ng/kgBW-day(37), [b]averages of two dose-response relationship between aflatoxin-albumin adduct and portions of stunting, number of children aged under 5 y.o. in 2010 is 23,600,000 (196)

6.4.2.1 Impacts of aflatoxin control interventions on stunted growth prevalence

Table 6-4. Potential impacts of aflatoxin control interventions on aflatoxin-related stunting burden in Nigeria (Upper bound level of aflatoxin)

	Biocontrol			The postharvest intervention package			NovaSil clay		
	Maize	Groundnuts	Total	Maize	Groundnuts	Total	Maize	Groundnuts	Total
AF exposure per kgBW-day (ng)	225.1	36.5	258.6	225.1	36.5	258.6	225.1	36.5	258.6
% aflatoxin-related stunting [a]	15.6%-21.6%			15.6%-21.6%			15.6%-21.6%		
Efficacy of intervention	80%	NA	NA	NA	69%	NA	40%	40%	40%
Total exposure/ kg-d in ng (treated)	45.0	36.5	80.5	225.1	11.3	236.4	135.1	21.9	157.0
Predicted % stunting (int+)	0%			10.2%-16.6%			0%-1.2%		
No. of cases prevented[b]	736,320 to 1,014,800			231,280 to 254,880			736,320 to 958,160		
DALYs averted	1,269,172 to 1,749,180			398,650 to 439,329			1,269,172 to 1,651,551		

Note: [a]based on two dose response relationships between AF-alb adduct and proportions of stunting. [b]the number of population age five years and under was divided by five to account for an annual cost. One stunting survivor = 0.23 DALYs, one dead case = 33.2 DALYs (354).

Table 6-4 presents the impacts of our selected aflatoxin control interventions on stunting burdens in Nigeria. If the stunting burdens prevented were included in the analysis, all interventions would become very cost-effective. This result suggests that aflatoxin would be responsible for a substantial number of public health burdens related to stunting in Nigeria.

At the upper bound level of aflatoxin, when the benefits of preventing stunting burdens were included, all interventions were very cost-effective (CERs <1 GDP). The costs to prevent one healthy life year lost were dramatically decreased when the benefits of preventing stunting were included compared with the CERs of these three interventions, when liver cancer prevention was the only outcome of interest. However, at the lower bound level of aflatoxin, there was no difference in the CERs of these three interventions, because at this low level, aflatoxin did not induce stunting. Hence, the burdens of aflatoxin do not increase linearly with exposure levels. At high doses, aflatoxin may induce other serious adverse effects, especially in sensitive populations. Though it can be impossible to completely get rid of aflatoxin, it is crucial to control aflatoxin exposure to the yet to be identified safe level.

Table 6-5. Cost-effectiveness ratio of aflatoxin control interventions by outcomes of interest

Outcome of interest	Cost to prevent one healthy life year lost ($)					
	Lower bound aflatoxin exposure			Upper bound aflatoxin exposure		
	BC	PH	NS	BC	PH	NS
Liver cancer	7,319	69,110	24,755	2,702	66,142	11,177
Liver cancer + Stunting	7,319	69,110	24,755	49	614	130

Note: BC= biocontrol, PH = the postharvest intervention package, NS = NovaSil clay

The question is, if more than one highly cost-effective intervention is implemented, whether the combinations are worth doing. To determine the cost to prevent one extra DALY of aflatoxin control intervention combinations, we performed an incremental cost-effectiveness analysis. This type of analysis is a tool to determine cost per one unit outcome gained compared to the next best available method.

6.4.3 Incremental Cost-Effectiveness

In our prior analysis, we determined the cost-effectiveness of three aflatoxin control strategies and treated them as independent of each other. This kind of analysis works in the situation for which only one intervention is adopted. But if there are no budget constraints, more than one intervention can be implemented to maximize benefits, as long as the combinations are worth the cost. The problem is that whether adding one more intervention makes the combination to be worthy to be implemented. We performed an incremental cost-effectiveness analysis to answer this question. The incremental cost-effective ration (ICER) in incremental cost-effectiveness analysis provides information on how much the cost is to provide one additional unit of outcome to switch from current intervention or from doing nothing to alternative practices. The incremental cost-effective ratio can be presented as follows:

$$ICER \equiv (Cost(A) - Cost(B)) \div (Efficacy(A) - Efficacy(B))$$

Equation 8: Incremental cost effectiveness ratio

We compared ICER of biocontrol, the most cost-effective intervention, and the combinations of biocontrol and the other two interventions as double intervention and triple intervention as follows:

1) Biocontrol
2) Biocontrol+ the postharvest intervention package
3) Biocontrol+ NovaSil clay
4) Biocontrol+ the postharvest intervention package+ NovaSil clay

6.4.3.1 Baseline aflatoxin exposure

Based on the Nigerian maize and groundnuts consumption rates (73.6 and 6.4 grams per person per day), the estimated daily aflatoxin exposures for a Nigerian from consuming aflatoxin contaminated maize and groundnuts are 44.34 and 7.22 ng/kgBW-day, respectively. In children, aflatoxin exposures from consuming aflatoxin-contaminated maize and groundnuts range from 82.0 to 222.15 ng/kgBW-day and from 35.0 to 36.46 ng/kgBW-day, respectively. Without any intervention, aflatoxin in maize and groundnuts combined would cause about 18.6% –an average

of upper and lower bound predicted values: 15.6% to 21.5% –stunting in Nigerian children age five years old and under (see table 6-3).

6.4.3.2 Impacts of combinations of aflatoxin control interventions on preventing stunting (Children age 0-5 years old)

With the application of biocontrol in maize fields, aflatoxin levels will be reduced to levels that cannot induce stunting in children, either in the presence or absence of other interventions. Numbers of stunting cases prevented and DALYs saved from the combinations of biocontrol and the other interventions (the postharvest intervention package and NovaSil clay) are similar to those of biocontrol alone, 875,560 fewer cases and 1,509,176 DALYs averted, respectively.

6.4.3.3 Impacts of combinations of aflatoxin control interventions on preventing liver cancer

Biocontrol (Scenario I): Please see table 6-5

Biocontrol + the postharvest intervention package (Scenario II): Biocontrol reduces aflatoxin in maize by 80%; whereas, the postharvest intervention package reduces aflatoxin in groundnuts by 69%, leaving 20% aflatoxin in treated maize and 31% aflatoxin in treated groundnuts. Overall, the combination of biocontrol and the postharvest intervention package would reduce aflatoxin exposure by 40.5 ng/kgBW-day. As a result, 3,290 aflatoxin-induced liver cancer cases, and 31,822 DALYs can be prevented.

Biocontrol + NovaSil clay (Scenario III): NovaSil will reduce aflatoxin bioavailability by 40% of aflatoxin contamination in foods. Some of them include biocontrol-treated maize and untreated groundnuts. Therefore, the combination of biocontrol and NovaSil clay can reduce total aflatoxin bioavailability in maize by 88% and bioavailability of aflatoxin in groundnuts by 40%. On the whole, biocontrol, along with NovaSil clay, would reduce aflatoxin exposure by 41.9 ng aflatoxin per kgBW per day. Thus, 3,408 aflatoxin-induced liver cancer cases and 32,965 DALYs will be averted.

Biocontrol + the postharvest intervention package + NovaSil clay (Scenario IV): Aflatoxin in contaminated maize would be reduced mainly through biocontrol and NovaSil clay. The overall aflatoxin reduction was 88% (80% from biocontrol and 40% of the remaining from NovaSil clay). Similarly, aflatoxin in groundnuts would be reduced by 81% (69% from the postharvest intervention and 40% of the remaining from NovaSil clay). The total aflatoxin prevented from consuming maize and groundnuts contaminated with aflatoxin would be 44.8 ng per kgBW-day. With this level of aflatoxin reduction, 3,649 liver cancer cases and 35,294 disability life years lost will be prevented.

Table 6-6. Marginal cost-effectiveness of some selected single or combinations of aflatoxin control strategies

Strategy	Cost (million $)	DALYs saved	Marginal cost (million $)	Marginal effectiveness	Ratio $/DALYs saved
BC	75.4	1,537,079	75.4	1,537,079	49
BC+PH	179.1 to 334.6	1,540,998	103.7 to 259.2	3,919	26,457 to 66,142
BC+NS[a]	263.3	1,542,141	187.9	5,062	37,109
BC+PH+NS	366.9 to 552.5	1,544,470	187.9	3,472	54,107

Note: BC: biocontrol, NS: NovaSil clay, PH: the postharvest intervention package, [a]The combination of BC and NS was compared with BC, not BC+PH, numbers of prevented stunting cases were divided by five to obtain the annual effect of intervention. One stunting survivor = 0.23 DALYs, one dead case = 33.2 DALYs (354)

As a stand-alone intervention, each intervention could be a worthy intervention, but if we want to get higher benefits by implementing more than one intervention, the combinations would not be worth since the new CERs are more than 3 Nigerian GDP.

To summarize, biocontrol, the postharvest intervention package, and NovaSil clay, as stand-alone interventions, are cost-effective; but our analyses indicate that if these interventions were combined, none of the combinations would be cost-effective. In other words, any extra health benefits associated with an additional intervention would be significantly smaller than the corresponding costs, in WHO cost-effectiveness terms. Combinations of biocontrol with either the postharvest intervention package or NovaSil clay provide extra benefits than biocontrol alone. However, the benefits gained are much fewer than the sum of the benefits from biocontrol and the other intervention. The reasons for the increased cost per DALYs prevented of the combinations are two-fold as follows:

1) Interventions act directly or indirectly to the same food commodities. (Biocontrol/NovaSil clay and the postharvest intervention package/ NovaSil clay)
2) Interventions act on food commodities taken in small amounts. For example, the postharvest intervention package, if applied to groundnuts only, would not be cost-effective, because Nigerian consume a much smaller amount of groundnuts compared with maize.

In the case of the combination of biocontrol and the postharvest intervention package, even if these two interventions were applied to different food commodities, biocontrol alone can reduce aflatoxin in maize to the levels that cannot produce stunting; therefore, implementing the postharvest intervention package to biocontrol-adopted areas does not produce extra benefits as far as reducing stunting risk. As a result, the extra benefits of this combination are due solely to the additional reduction of aflatoxin-induced liver cancer burdens from the reductions of aflatoxin in groundnuts. Similarly, no extra stunting prevention benefits from adopting NovaSil clay would be expected in a region, in which biocontrol is already 100% adopted. Furthermore, the efficacy of NovaSil clay is affected by the presence of biocontrol, as previously mentioned.

6.4.4 Sensitivity analysis

We determined sensitivity of the cost-effectiveness ratio by varying either costs or efficacy of the interventions. For each intervention, we varied its efficacy from 0.4 to 1.6 times the original efficacy or until maximum efficacy (100%) was achieved. We varied the costs of intervention upward to determine the maximum cost in the terms of times the original costs that brings the CER beyond three times GDP per capita, the threshold for cost-effectiveness analysis.

Table 6-7. Cost effectiveness ratios of biocontrol

		Multipliers of original cost							
CER =	49	0.5	1	5	10	20	50	100	200
Multipliers of original efficacy	0.4	32	63	316	633	1,265	3,164	6,327	12,654
	0.6	25	49	247	494	988	2,471	4,941	9,883
	0.8	25	49	246	492	985	2,462	4,923	9,847
	1	25	49	245	491	981	2,453	4,905	9,811
	1.12	24	49	245	489	979	2,447	4,895	9,789

Table 6-8. Cost effectiveness ratios of the postharvest intervention package

		Multipliers of original cost							
CER =	614	0.5	1	5	10	20	50	100	200
Multipliers of original efficacy	0.4	766	1,532	7,660	15,320	30,641	76,601	153,203	306,406
	0.6	511	1,022	5,111	10,222	20,444	51,110	102,220	204,441
	0.8	383	767	3,835	7,670	15,339	38,349	76,697	153,395
	1	307	614	3,069	6,137	12,275	30,687	61,373	122,746
	1.2	256	512	2,558	5,115	10,231	25,576	51,153	102,306

Table 6-9. Cost-effectiveness ratios of NovaSil clay

		Multipliers of original cost							
CER =	130	0.5	1	5	10	20	50	100	200
Multipliers of original efficacy	0.4	143	285	1,426	2,851	5,702	14,255	28,510	57,021
	0.6	95	190	951	1,901	3,802	9,505	19,011	38,022
	0.8	71	143	713	1,426	2,852	7,130	14,260	28,519
	1	65	130	648	1,297	2,594	6,484	12,968	25,935
	1.2	65	129	647	1,294	2,588	6,469	12,938	25,875
	1.4	65	129	645	1,291	2,582	6,454	12,908	25,815
	1.6	64	129	644	1,288	2,576	6,439	12,878	25,756

6.5 DISCUSSION

A threshold level of aflatoxin on growth development is one of the most important features of the dose-response relationship between aflatoxin exposure or its biomarkers and growth performance. Aflatoxin exposure must be high enough to deteriorate growth to the extent that a particular growth impairment defined (HAZ, WAZ, or WHZ <-2SD). The threshold of AF-alb derived from the Benin and Togo study is about 16.7 pg/mg albumin. Based on the same study, once the threshold level of aflatoxin exposure to induce stunting was reached, the prevalence of stunting will increase by 1.96 to 2.12 per 100 per picogram of the adduct/mg albumin.

The stunting burdens caused by aflatoxin contamination would be much higher than burdens from aflatoxin-related liver cancer. Whereas, the prevalence of liver cancer is per 100,000 basis, the prevalence rate of stunting is based on 100 c hildren age between 0-5 years old. For a country in which birth rate is usually high and the number of elder population is relative low, compared with those of adolescents and adults, such as Nigeria, public health burdens from stunting can be several times higher than that of liver cancer. Burdens of aflatoxin-related stunting in Nigeria can be significant high more than previously expected that if preventing stunting was included in the analysis, it turned the postharvest intervention package from not cost-effective to be very cost-effective.

When benefits from preventing stunting are included, all of these four interventions as a single intervention are very cost-effective. Choosing a particular intervention over the others depends on several factors. Biocontrol, in our analysis, is the most cost-effective intervention in reducing aflatoxin toxicities. But in some circumstances that biocontrol cannot be performed– for example, atoxigenic fungi have not been identified in that particular area–NovaSil clay or the postharvest intervention package could be an alternative intervention. These three aflatoxin specific control interventions and hepatitis B vaccine reduce different risk factors of liver cancer; therefore, they can be considered as add-on interventions of each other.

7.0 EVALUATING THE TECHNICAL FEASIBILITY OF AFLATOXIN RISK REDUCTION STRATEGIES IN AFRICA

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Felicia Wu, Pornsri Khlangwiset

Department of Environmental and Occupational Health, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA USA

7.1 ABSTRACT

Public health interventions must be readily accepted by their target populations to have any meaningful impact, and must have financial and infrastructural support to be feasible in the parts of the world where they are most needed. At the same time, these interventions must be assessed for potential unintended consequences, either to the environment or to human health. In this study, we evaluate the technical feasibility of interventions to control aflatoxin risk, to be potentially deployed in parts of Africa where aflatoxin exposure poses a significant public health concern. We apply a conceptual framework for feasibility to four interventions, one associated with each of four different stages of aflatoxin risk: biocontrol (preharvest), a postharvest intervention package (postharvest), NovaSil clay (dietary), and hepatitis B vaccination (clinical). For each intervention, we assess the following four components of technical feasibility: 1) characteristics of the basic intervention, 2) characteristics of delivery, 3) requirements on government capacity, and 4) usage characteristics. We describe ways in which feasibility of each intervention is currently high or low from the perspective of adoption in Africa, how public education is crucial for each of these interventions to succeed, and how to align economic incentives to make the interventions more suitable for less developed countries.

7.2 INTRODUCTION

Aflatoxin, produced by the fungi *Aspergillus flavus* and *A. parasiticus* on crops such as maize, peanuts, and tree nuts, is recognized to be an important food safety risk worldwide. Aside from causing acute poisoning at high doses (acute aflatoxicosis), aflatoxin can also cause liver cancer (hepatocellular carcinoma, or HCC), immunomodulation (10, 23, 348, 356), and stunted growth in children at chronic lower-level doses (22, 27, 35). More recent evidence shows that aflatoxin may also play a role in global cirrhosis morbidity (357). Most of these health problems associated with aflatoxin exposure occur in less developed countries (LDCs) in tropical and

subtropical areas of the world, where the Aspergilli thrive and where food safety measures are less stringent.

An interesting aspect of the aflatoxin public health issue is that the risk can be mitigated at so many different levels, in multiple different ways. This stands in contrast to other foodborne risks such as harmful bacteria (e.g., *Escherichia coli*): no enterosorbents could reduce their levels in the gastrointestinal system, and no vaccines could mitigate their impacts. Aflatoxin accumulation could be reduced in crop fields, in food storage, or in food processing. Additionally, even if aflatoxin is present in consumers' food, certain dietary additives or clinical practices can mitigate adverse effects of the toxin in the body. Hence, many different interventions have been developed either to reduce aflatoxin directly in the field and in food (preharvest and postharvest interventions), or to reduce aflatoxin's harmful effects in the body once it is ingested (dietary and clinical interventions). These categories of interventions are described in greater detail in Khlangwiset and Wu (2009) and Wu and Khlangwiset (2009).

Regulations on maximum allowable levels of aflatoxin in food could also reduce aflatoxin exposure. These regulations are generally effective in controlling aflatoxin in industrial nations; commodities that contain aflatoxin levels exceeding regulatory guidelines for human food or animal feed are discarded, or sold at a lower price for a different use (38, 39). However, aflatoxin regulations in many LDCs do little to protect public health, as there is limited enforcement of food safety regulations, especially among rural communities where food quality is rarely formally inspected (37). Subsistence farmers and local traders sometimes have the luxury of discarding obviously moldy maize and groundnuts. But in drought seasons, people often have no choice but to eat moldy food or starve. Thus, regulations do little to help reduce aflatoxin and its related health effects in LDCs (37, 358). Rather, the focus should be on promoting adoption of strategies that can control aflatoxin and its associated risks, in the field, in postharvest conditions or in the diet (135).

This dichotomy between the feasibility of aflatoxin regulations against the feasibility of other kinds of public health interventions highlights the need for mycotoxin researchers to consider whether the control strategies they develop could actually be implemented widely to improve public health. It is crucial that an intervention be technically feasible in the places where it is most needed. The purpose of this paper is to highlight key components of technical

feasibility, which are then applied to analyzing four specific interventions that control aflatoxin risk.

7.3 FRAMEWORK TO CONSIDER FEASIBILITY OF AFLATOXIN CONTROL STRATEGIES

The cost-effectiveness of selected public health interventions to control aflatoxin-induced liver cancer has been assessed (135). Yet cost-effectiveness of a strategy is not enough to ensure its successful adoption. More questions must be posited, such as: Are there countervailing health or ecological risks to the strategy? What would the delivery mechanism be, and would locally-available infrastructures support the mechanism? Do governmental regulations inhibit or promote the intervention? Is the intervention culturally appropriate and easy to adopt by the target population (135). If an intervention to reduce aflatoxin fails in any or all of these points, then it is not likely to be adopted on a global scale, no matter how cost-effective it may be.

A conceptual framework to evaluate the technical complexity – and hence the feasibility – of public health interventions for less developed countries has been developed (359). There are four relevant dimensions (see figure 7-1):

1. Characteristics of the basic intervention
2. Characteristics of delivery
3. Requirements on government capacity
4. Usage characteristics

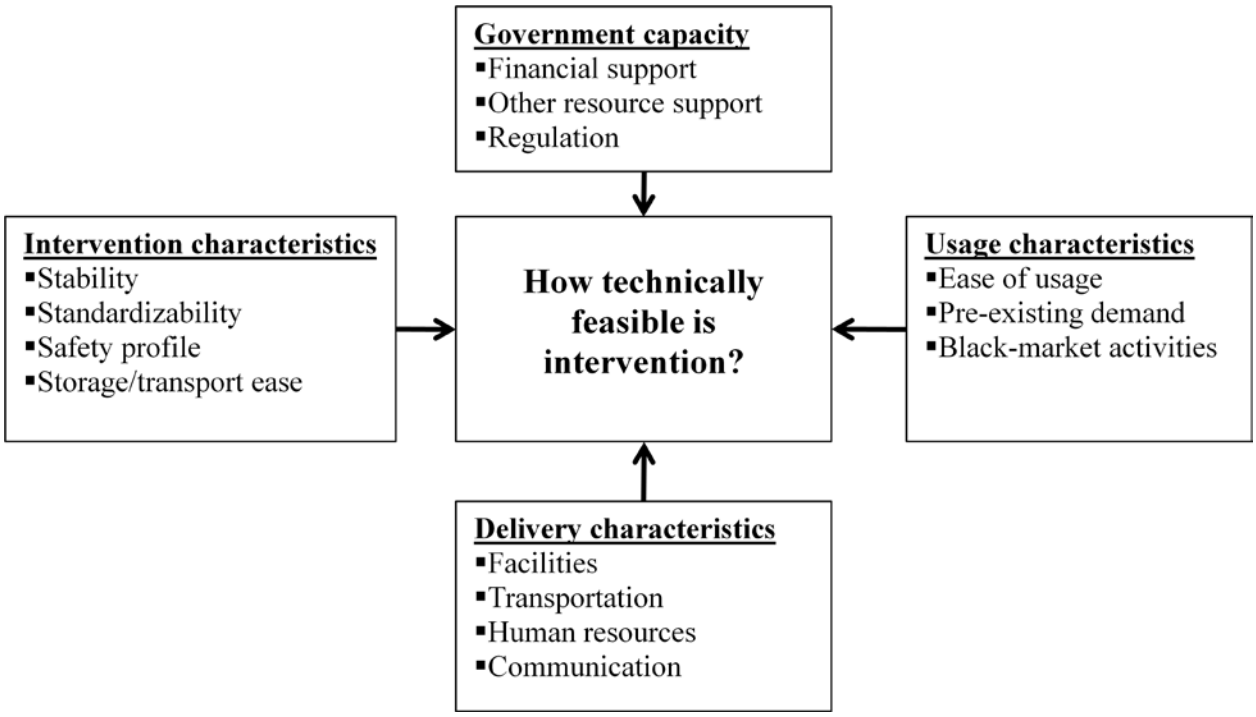

Figure 7-1 Framework to assess technical feasibility of public health interventions.

7.3.1 Aflatoxin control strategies

Multiple public health interventions exist by which to control aflatoxin or its burden in the body, to prevent HCC. Several of these are listed in Table 1, adapted from Wu and Khlangwiset (2010). We are beginning to understand more about the cost-effectiveness of these interventions; what remains to be found is how technically feasible they would be in many parts of the world.

Table 7-1. Sampling of interventions to reduce aflatoxin risk in field, dietary, and clinical settings

Setting		Intervention
Agricultural	Preharvest	▪ Suitable hybrid choice ▪ Transgenic or conventional breeding for plant host resistance ▪ Biocontrol ▪ Chemical control (insecticides, fungicides) ▪ Good agricultural practices ▪ Antioxidants (e.g., caffeic acid, gallic acid)
	Postharvest	▪ Cleaning ▪ Mechanical sorting and segregation ▪ Improved storage / drying / transportation conditions ▪ Ammoniation ▪ Ozonation ▪ Chemical control (insecticides, fungicides)
Dietary		▪ Enterosorbents (e.g., calcium aluminosilicates, glucomannan, chlorophyllin) ▪ Chemopreventive agents (e.g., Oltipraz, isothiocyanates, triterpenoids) ▪ COX-2 inhibitors ▪ Green tea polyphenols
Clinical		▪ HBV vaccination

Interventions to reduce aflatoxin risk can be roughly grouped into three categories: 1) agricultural (preharvest and postharvest), 2) dietary, and 3) clinical. *Agricultural* interventions are methods or technologies applied either in the field (preharvest) or in drying, storage and transportation (postharvest) to reduce aflatoxin levels in food. Agricultural interventions can thus be considered "primary" interventions, because they can reduce actual aflatoxin levels in food. *Dietary* and *clinical* interventions can be considered "secondary" interventions. They cannot reduce aflatoxin levels in food, but can ameliorate aflatoxin-related illness; by reducing bioavailability either of aflatoxin (e.g., through enterosorption) or of its reactive oxygen species

that binds to DNA to initiate cancer (e.g., through induction of Phase 2 enzymes that detoxify the aflatoxin-8,9-epoxide).

We assess the technical feasibility of one intervention from each of these categories, specifically for human use:

1. Biocontrol (preharvest)
2. A postharvest intervention package (postharvest)
3. Calcium aluminosilicate clay (NovaSil) as an enterosorbent (dietary)

Hepatitis B vaccine is included in our analyses, even though it is not literally considered as an aflatoxin control intervention. The vaccine itself has no impact on actual aflatoxin levels in diets, but it prevents the synergistic impact of HBV and aflatoxin in inducing liver cancer.

For each of these, we evaluate characteristics of each intervention according to the aforementioned dimensions of the intervention's basic characteristics, delivery characteristics, government capacity requirements, and usage characteristics. In addition, we describe economic issues associated with wide-scale adoption of each intervention. We describe the areas in which the characteristics of each intervention lend themselves to being more or less feasible in LDCs, with a focus on sub-Saharan Africa, where these interventions have shown success in field and clinical trials.

7.4 APPLICATION OF TECHNICAL FEASIBILITY FRAMEWORK TO AFLATOXIN RISK-REDUCTION INTERVENTIONS

7.4.1 Biocontrol: Technical feasibility

Agricultural biocontrol involves the use of biological agents to control pests or toxin production. Specifically, biocontrol of aflatoxin refers to using organisms to reduce the incidence of toxigenic Aspergilli in susceptible crops, and thereby to reduce aflatoxin contamination. The most widely used biocontrol method for aflatoxin employs nontoxigenic strains of Aspergilli that can competitively exclude toxigenic strains from colonizing crops. Grain seeds (of wheat, barley, sorghum, or other small grains) are either briefly colonized by or coated with conidia of a

nontoxigenic strain, and these seeds are applied to agricultural fields during a period favorable for competitive exclusion of toxigenic strains.

These biocontrol methods have been used in maize, groundnuts, and cottonseed in several regions of the world (57-62).Importantly, nontoxigenic *A. flavus* strains have been found in sub-Saharan Africa, which show promise for controlling aflatoxin in African maize (59, 62). Biocontrol methods, though applied in the field, can result in reduced aflatoxin in crops for months postharvest (360).

Table 7-2 summarizes the characteristics of biocontrol as an aflatoxin reduction intervention in African countries that determine its overall feasibility.

Table 7-2. Technical feasibility characteristics of biocontrol for aflatoxin control in Africa

Category	Criteria	Intervention
Intervention Characteristics		
Basic product design	Stability	Shelf life ~ 6 months; dependent on temperature and moisture control
	Standardization	Needed to ensure that each application unit of biocontrol contains sufficient amounts of living nontoxigenic fungi to competitively exclude toxigenic fungi
	Safety profile	Low risk of inhalation aspergillosis and skin and eye irritation; minimal risk of toxicity and infectivity
	Ease of storage and transportation	Must be stored and transported at low relative humidity and avoiding either temperature extreme
Supplies	Need for regular supplies	Nontoxigenic spores must be maintained in cultures, and grains must be provided regularly as a substrate
Equipment	High-technology equipment and infrastructure needed	Equipment to manufacture and maintain fungal spores, sterilize substrate, and mix spores and substrate with a binder

Table 7-2 (continued)

Category	Criteria	Intervention
Delivery characteristics		
Facilities	Retail sector	Needed if biocontrol application is done by farmers
	Outreach services	Monitoring proportion of nontoxigenic spores in the field to ensure continued effectiveness Educating growers on why aflatoxin is an important problem, and how to optimally apply biocontrol
	Laboratories	See "Equipment" above
Human resources	Trained scientific professionals	Professional staff to produce and maintain nontoxigenic spores, operate equipment to produce all parts of biocontrol, and to apply it (in situations where farmers cannot themselves)
	Outreach staff	Community volunteers, agricultural and health care providers to highlight the importance to control aflatoxin in food and feed stuffs. Laboratory workers to routinely monitor aflatoxin levels in agricultural goods. Agricultural extension service to provide advice, suggestion or recommendation on biocontrol.
Communication and transport	Dependence of delivery on communication and transport infrastructure	Crucial for biocontrol to reach diverse parts of African countries where maize and other aflatoxin-vulnerable crops are planted
Government capacity requirements		
Regulation/ legislation	Need for regulation	Need for biopesticide registration in African countries
	Need for monitoring and enforcement	Need for aflatoxin monitoring in agricultural goods Need for monitoring potential health effects produced by biocontrol agents (e.g., skin irritation, aspergillosis) Need for monitoring potential changes in toxigenicity of nontoxigenic fungi

Table 7-2 (continued)

Category	Criteria	Intervention
Collaborative action	Collaborative efforts within government sectors and between government and other groups	Collaborations among ministries of agriculture and health could provide a stronger basis for biocontrol as a means of improving public health through farming technologies. International organizations (e.g., IITA, WHO, Foundations) have made, and should continue to make, important contributions toward aflatoxin reduction in Africa (funding, staff, etc.).
Usage characteristics		
Ease of usage	Need for information, education, supervision	If biocontrol application in hands of farmers: instructions can be printed on packages, agricultural outreach staff can assist and supervise in some areas. More broadly, education is needed to inform farmers why aflatoxin is an important problem and how biocontrol can reduce risk.
Pre-existing demand	Need for promotion	Little if any pre-existing demand for biocontrol in most African nations; promotion essential among farmers
Black-market risk	Need to prevent counterfeiting	Counterfeiting is possible but unlikely: toxigenic fungi could be packaged and sold as biocontrol to farmers

7.4.1.1 Biocontrol: Basic characteristics.

Three main elements are important to consider here: the basic product design (stability, standardization, safety profile, and ease of storage and transport), supply requirements, and equipment needed. Biocontrol agents have a shelf life in the United States of up to six months (361), which may be lower in the tropics depending on quality of storage conditions, including susceptibility to insect damage. Standardizing biocontrol is highly important, to ensure that each application unit contains sufficient amounts of nontoxigenic fungi to competitively exclude toxigenic strains in the field.

Safety is an important consideration regarding biocontrol use in Africa. Because of the potential risk of invasive aspergillosis from *A. flavus* exposure among immunocompromised individuals (as highlighted in Krishnan et al. 2009 and Hedayati et al. 2007), it is important to ensure that the biocontrol material is manufactured and applied in such a way as to minimize

direct inhalation of spores. The biocontrol methods used commercially and in field trials in various parts of the world, in which the nontoxigenic Aspergilli are applied in an oil or molasses mixture to seeds (60, 61) that are then applied to fields, minimize potential inhalation risks. At the present time, no aspergillosis cases have been reported as a result of biocontrol manufacturing or application. In the United States, it is recommended that applicators wear protective gear – a long-sleeved shirt, long pants, shoes, sock and gloves−when working with biocontrol (362), because of concerns regarding potential moderate eye or skin irritation, though no cases have thus far been reported (363). These precautions should be followed in other parts of the world where biocontrol is used.

Several conditions are necessary for optimal storage and transport. Grains colonized by nontoxigenic Aspergilli, once dried, should be kept in moisture-protected, insect-proof bags, which should not be exposed to high relative humidity or extreme temperatures, such as over 80% RH, over 50° C, or below freezing (363). In most parts of Africa, these temperature constraints would pose no problem; however, relative humidity could be higher than optimal for storage of biocontrol agents.

High-technology equipment, basic supplies, and trained professional staff are necessary to produce and maintain biocontrol agents: to maintain the cultures used, to manufacture the large numbers of nontoxigenic fungal spores needed for application to the substrate (the grains used to convey the spores in the field), to sterilize the substrate on a large scale, and to mix the spores and substrate with a binder (such as oil or molasses) to allow the spores to adhere (60, 135). The ability of African countries to meet these high-technology requirements varies from nation to nation. Nigeria would benefit from the support of the Ibadan-based International Institute of Tropical Agriculture (IITA), with an active research and outreach group for biocontrol adoption. Biotechnology has also flourished in certain African countries such as Kenya, Zimbabwe, Egypt and South Africa (364). These countries may thus be more capable of producing biocontrol agents than others, although training needs for biocontrol specifically may be minimal

There are potential supply constraints as well. The substrate itself, such as wheat, rice, or sorghum grains (365), must be available; this may be problematic to obtain in situations of food insecurity, when the grains would be better used as a food source rather than as a substrate to convey biocontrol agents. Maize cobs have been proposed as a potential substrate on which

nontoxigenic fungi can be applied and then dispersed in fields (59), which would alleviate grain supply concerns.

7.4.1.2 Biocontrol: Delivery characteristics.

Specifics of biocontrol delivery in Africa may vary by nation. There may be centralized laboratories and facilities to provide the biocontrol materials; or the facilities may be more widely distributed within the target population, particularly if individual farmers will apply the biocontrol to their fields. If farmers are already using agricultural chemicals such as pesticides and fertilizers, the biocontrol agents could be obtained through the same venues.

There are benefits and costs associated with both centralization and dispersion of facilities to produce and maintain biocontrol, and individuals who apply biocontrol to fields. For example, transportation costs to get biocontrol materials to the places they are needed are lower if the facilities are more dispersed within a community; but having a greater number of facilities necessitates having trained professionals at each location, which may cause a strain on human resources. Likewise, hiring trained professionals to apply biocontrol to crop fields would ensure better quality control of application. However, it leads to questions of who would pay for and train these professionals, and whether there would be a sufficient number of them to ensure that the biocontrol was applied at exactly the right time to allow competitive exclusion of aflatoxin-producing fungi in the field. Ultimately, it should be the individual farmers who apply biocontrol, with guidance either from a local agricultural extension worker or from the biocontrol packaging materials.

7.4.1.3 Biocontrol: Government capacity requirements.

Two items should be considered: governmental regulations on use and commercialization of the intervention, and regulatory support and enforcement to ensure optimal benefits and minimal risk. Biocontrol for aflatoxin reduction was first registered in the United States, by the Environmental Protection Agency (366). A lengthy review ensured no evidence or likelihood of risks to human health or to the environment. The review included tests with laboratory mammals to assess oral or lung infectivity, toxicity, and allergenicity; soil and air monitoring studies for environmental quality; survival tests of the fungi after crop processing, and ecological risk

assessments of endangered species encountering the fungi. In all cases, risks were shown to be minimal or nonexistent (61).

With this precedent to guide further health and environmental risk assessments in different parts of the world, governmental regulatory barriers may be minimal. The International Institute of Tropical Agriculture (IITA) has held workshops aiming to create harmonized biopesticide regulation for African countries. The biopesticide regulation system within the member countries of the Permanent Interstates Committee for Drought Control in the Sahel (CILSS) has been proposed as a protocol of harmonized regulation for biopesticide registration. Pesticides registered by CILSS can be used in any CILSS member nations (367). If this approach is developed and accepted by African countries, biocontrol application in Africa could be implemented on a wide scale. Moreover, stakeholder involvement in select nations has been mobilized. Recently, a stakeholder consultative meeting on biological control was held in April 2009 at Ibadan-based IITA, Nigeria; to discuss biocontrol application in maize to lower aflatoxin levels, as well as its potential usefulness in other staple crops (368).

Monitoring should also be conducted regularly, to ensure that the nontoxigenic strains do indeed continue to produce no aflatoxin in field conditions. Recently, an inexpensive aflatoxin test kit was developed by IITA and ICRISAT: a mere $1−$2 per analytical sample. This kit is purported to be effective even for analyses in the most remote rural areas of Africa (369).

7.4.1.4 Biocontrol: Usage characteristics.

The three aforementioned considerations regarding the end user - the ease of usage, pre-existing demand, and black-market risks – are legitimate concerns regarding widespread biocontrol adoption. Eventually, individual farmers must be the ones to apply biocontrol in African crop fields to control aflatoxin. The education and outreach process could be complicated – both in convincing the farmers of the need for this product, and in providing guidance on how much and when to optimally apply the biocontrol (61). There is little if any pre-existing demand for this product specifically, as it is a new technology; although there is pre-existing demand more generally for aflatoxin control. Finally, it is possible that black markets may arise that provide materials that are not truly nontoxigenic. This is why monitoring processes and quality control for marketed products are extremely important.

7.4.1.5 Biocontrol: Economic issues.

The low cost of biocontrol ($10-$20 per hectare) and its effectiveness at aflatoxin reduction (50-90%) make biocontrol a very cost-effective intervention in reducing aflatoxin-induced disease (135). However, individual farmers may not have incentive to pay even this relatively small amount if they do not understand the risks of aflatoxin, and moreover, have very little discretionary monies.

Therefore, initially, governments (in partnership with internal and external funding agencies) would most likely need to provide the resources for widespread biocontrol application. Alternatively, governments and/or commodity industries could establish a marketing system that provides a premium to growers for low aflatoxin levels (or penalizes high aflatoxin levels, which may be less successful). This system would provide economic incentives for the growers to pay for biocontrol, and is, most likely, the rational long-term approach. Meanwhile, public education on the health effects caused by aflatoxins and the method to manage aflatoxins at the field level must be provided regularly in order to encourage people to adopt this new technology, and change their behavior to protect themselves from aflatoxins exposure.

7.4.2 Postharvest intervention package: Technical feasibility

In industrial nations, food storage and processing practices usually prevent postharvest development of mycotoxins, but postharvest mycotoxin accumulation remains a threat in many LDCs. Hence, attention to key critical control points during harvesting, drying, and storage of food is essential, to reduce postharvest aflatoxin in LDCs (63, 64). Reducing postharvest aflatoxin accumulation can begin with simple physical methods. Mechanical sorting can separate aflatoxin-contaminated kernels from relatively cleaner ones, and proper drying can further reduce risks. To prevent the growth of Aspergilli in food storage, it is necessary to control moisture, temperature, and pests (66).

Combinations of these methods to reduce postharvest aflatoxin have been tested for efficacy in actual storage conditions. Turner et al. (2005) describe a postharvest intervention package to reduce aflatoxin in groundnuts, tested in Guinea. The package consisted of six

components: education for groundnut farmers on hand-sorting nuts, natural-fiber mats for drying the nuts, education on proper sun drying, natural-fiber bags for storage, wooden pallets on which to store bags, and insecticides applied on the floor of the storage facility under the wooden pallets.

After five months in the Guinea groundnut intervention study, individuals who had received and practiced the postharvest intervention package had on average 57.2% lower aflatoxin-albumin concentrations in the blood (8 pg/mg), compared with individuals in the control group (18.7 pg/mg; Turner et al. 2005). Indeed, the adduct levels in the intervention group after five months was similar to the adduct levels in both groups immediately postharvest, while the average adduct level in the control group increased by over 100%. Because this biomarker can be directly correlated with aflatoxin exposure in the diet (370), the results of the Guinea study imply that the postharvest intervention package could essentially prevent aflatoxin from accumulating beyond its immediate postharvest level, even after five months of storage. We evaluate the technical feasibility of the entire package in this study.

Table 7-3 summarizes the characteristics of the postharvest intervention package as an aflatoxin reduction intervention in African countries that determine its overall feasibility.

Table 7-3. Technical feasibility characteristics of postharvest intervention package for aflatoxin control in Africa

Category	Criteria	Intervention
Intervention Characteristics		
Basic product design	Stability	Drying/storage materials could last for 3-4 years if kept properly
	Safety profile	Extremely safe. Only potential health risk concerns insecticide use: not expected to be a problem if already locally available and a familiar product to farmers.
	Ease of storage and transport	Most raw materials are locally available; fiber mats and bags and pallets may need to be stored away from moisture and pests to extend lifetime
Supplies	Need for regular supplies	Drying/storage materials could last for 3-4 years if kept properly
Equipment	High-technology equipment needed	None; entire intervention relies on c ommunity-based technology and materials
	Maintenance needed	Fiber bag, mat and wooden pallet can become contaminated with fungi; sun drying and proper storage after use may reduce risk
Delivery characteristics		
Facilities	Retail sector and outreach services	If drying and storage materials are not made by each household, large scale production of these materials could be an option (e.g., for pallets). Local retail stores could provide the finished mats, bags, and pallets.
Human resources	Skill level required for service provision	Community volunteers/ agricultural extension staff or local agricultural authorities, to educate growers on the risks of aflatoxin and the methods of using the complete intervention to reduce aflatoxin

Table 7-3 (continued)

Category	Criteria	Intervention
Government capacity requirements		
Regulation / legislation	Need for regulation	No special regulation required
Collaborative action	Collaborative efforts within government sectors and between government and other groups	Collaboration between health and agricultural sectors, as well as between national and local level governments, is important. Outreach staff are an important part in this community based intervention. Funding from external agencies may be desirable to offset the initial costs of the packages.
Usage characteristics		
Ease of usage	Need for information/education	While the need for information and education is high (e.g., hand-sorting, drying, specific storage requirements), usage itself should be simple because of the cultural familiarity of the overall practices
Pre-existing demand	Need for promotion	Though the practices of drying and storage are familiar, the specifics (e.g., wooden pallets, fiber mats and bags) may not be, and growers may not understand the need for them. Hence, the need for promotion is crucial.
Black-market risk	Counterfeit prevention	Low risk of counterfeiting

7.4.2.1 Postharvest intervention package: Basic characteristics.

A beneficial feature of the postharvest intervention package is that most aspects of it are simple modifications of already-existing, culturally appropriate practices. Groundnut growers throughout Africa are already employing various methods to dry and store – and even apply insecticides to – groundnuts. The intervention builds upon what is already being done, with a specific goal of reducing aflatoxin accumulation.

Hence, the package's basic characteristics are simple and not substantially different from those of current practices for postharvest groundnut treatment in Africa. The recommended drying and storage materials (natural fiber mats and bags, wooden pallets) could last 3 to 4 years

if kept properly (Dr. Christopher Wild, personal communication). The mats and bags are, however, susceptible to mold and toxin contamination if not dried and stored properly; and people may use the wooden pallets for firewood or other uses if wood is scarce. The materials themselves are generally safe; the only potential health risk is through exposure to the insecticide. There is not likely to be additional risk, as insecticide recommendations for this package are based on what is already being used by growers. Most raw materials are locally available (67).

7.4.2.2 Postharvest intervention package: Delivery characteristics.

In order to deliver the postharvest intervention package to groundnut growers, provisions must be made for both facilities and human resources. As described above, wooden pallets should be custom-made and sold or distributed at local markets. Natural-fiber mats and bags can likely be purchased locally or made at home, with materials from cloth retailers.

A critical issue in the success of the postharvest package is public education. Hence, the human resource requirement is possibly the most important aspect of this intervention, as well as potentially the most difficult. Farmers must be shown how to identify groundnuts that are visibly moldy or damaged, and to discard them before storage. They must be shown how to judge the completeness of sun drying (on fiber mats) by shaking the kernels to listen for the free movement of the dried nuts. They must also be educated on the proper way to store the dried nuts: in natural fiber bags, on wooden pallets, with insecticide spread underneath (Turner et al. 2005). A substantial network of agricultural extension workers is needed to provide this education in rural groundnut-growing villages of Africa, to ensure the broader adoption that can lead to population health benefits. With proper training from extension staff, individuals in communities may be able to educate and train other farmers in their communities to apply the postharvest intervention package properly. It is crucial to develop community interest and support for such an intervention to succeed.

7.4.2.3 Postharvest intervention package: Government capacity requirements.

Presuming that the insecticides used are already registered in the target countries, no special regulation is required for wide-scale adoption of the intervention package anywhere in Africa.

Collaborative efforts between health and agricultural sectors would likely be beneficial in the efforts to educate groundnut growers throughout the nation, and to provide the necessary materials where growers would not be able to afford them. Funding from external agencies may be desirable to aid in the public education efforts, as well as to offset the initial costs of the packages.

7.4.2.4 Postharvest intervention package: Usage characteristics.

As described above, this intervention would rely heavily on user knowledge of and adherence to practices that reduce aflatoxin in postharvest conditions. Fortunately, growers are already practicing many of these post-harvest activities (sorting, drying, storage) in some form; it is a matter of optimizing the activities to reduce aflatoxin accumulation. The activities included in this intervention are culturally appropriate for many rural groundnut growers in Africa. There may be, however, difficulties in changing current practices. A previous study on a different postharvest intervention showed that only 6.3% of farmers in the Southern Guinea Savannah adopted an improved "crib" storage structure for crops, recommended by the Food and Agriculture Organization (371), though it was promoted in those communities. Behavioral change, even if beneficial, may be slow among communities.

7.4.2.5 Postharvest intervention package: Economic issues.

As with biocontrol, the main challenge to widescale adoption of the postharvest intervention package is providing the right economic incentives. Individual groundnut growers need the motivation to undergo the education and all the actions and costs needed to implement this package, which can be difficult if aflatoxin is not recognized as a significant public health or market problem. In this case, unlike biocontrol, the package cannot be applied by agricultural staff going from household to household; the growers themselves must implement the intervention. Moreover, the total package was estimated in 2005 to cost $50 per household (67). The wooden pallet, the largest cost in the total intervention package, is the most difficult part for groundnut growers to be able to make on their own. These must be purchased or otherwise distributed from retail outlets.

Economic issues of a different nature concern the incentives of poor growers who do not understand or seriously regard the extent of aflatoxin-induced illness. First, one must consider the fate of groundnuts sorted out because of high aflatoxin levels. Even if growers are trained to do this with a high degree of accuracy (as part of the postharvest intervention package), it is not known what would happen to those contaminated nuts. If they are kept out of the marketplace, then indeed, consumers who can afford to buy nuts from markets will be better-protected. But if they are consumed by poor households who cannot afford to discard the nuts, then the poorest people in Africa would still suffer the greatest burden of aflatoxin-induced risk. Second, if wood is a scarce resource in poor households, the wooden pallets may be destroyed for alternative uses (such as firewood) rather than used for their intended purpose: to elevate the stored groundnut bags for postharvest protection against aflatoxin accumulation.

Hence, as part of this intervention package, public education on health risks of aflatoxin is absolutely crucial, to ensure the right economic and health incentives for groundnut growers to adopt the intervention and to remove highly contaminated nuts from the human food chain.

7.4.3 NovaSil: Technical feasibility

A variety of dietary interventions can reduce aflatoxin-related health risks. One simple dietary intervention, where feasible, is to consume relatively less maize and groundnuts, in favor of other food crops that usually have lower aflatoxin levels such as sorghum and pearl millet (69). Where it is not easy to make such a conversion, however (e.g., where maize and groundnuts have traditionally been staples in the diet), other dietary interventions may prove helpful. Dietary additives to reduce aflatoxin-induced risk include enterosorbents that "trap" aflatoxin in the gastrointestinal (GI) tract, facilitating elimination (70, 78); agents that induce Phase 2 enzymes to conjugate aflatoxin's reactive oxygen species in the liver (74, 85, 86); or anti-inflammatory agents (76, 105).

We focus on NovaSil clay, an enterosorbent of aflatoxin. Enterosorbents can be blended into food or feed, or taken separately (e.g., in capsule form) during mealtimes to bind aflatoxin in the GI tract, resulting in reduced aflatoxin bioavailability in the body. Several materials have varying degrees of this ability to bind aflatoxin, including bentonites, zeolites, diatomaceous

earth, activated charcoal, yeast cell walls, and fibers from plant sources. One material that has proven effective in animal feed and is showing promise in human trials is calcium montmorillonite, marketed as NovaSil clay (NS). NS has been shown to prevent aflatoxicosis in many animal species when included in their diet, by binding aflatoxin with high affinity and high capacity in the GI tract (70). Importantly, in humans, aflatoxin-albumin adducts in both low-dose and high-dose NS intervention arms were significantly lower than those in the control arm after three months, with a roughly 25% reduction: 0.89-0.90 pmol/mg adducts in albumin compared to 1.20 pmol/mg in the control arm. However, only the high- dose group shows the significant lower levels of AFM1 after three months (104).

One advantage of including NS (or other effective enterosorbents) in a comprehensive plan to reduce aflatoxin risk is that it can mitigate adverse health effects even if preharvest and postharvest conditions were conducive to high aflatoxin levels in food. NS could conceivably be used in "emergency" situations when aflatoxin levels are determined to be high in foodstuffs – by then, it is too late to change preharvest or postharvest practices to improve the food available to people at that moment, and few other options to reduce aflatoxin risk are possible. While NS does not directly reduce aflatoxin levels in food, it can reduce aflatoxin bioavailability. The feasibility of including NS as an aflatoxin risk-reduction intervention is summarized in table 7-4.

Table 7-4. Technical feasibility characteristics of NovaSil clay for aflatoxin risk reduction in Africa

Category	Criteria	Intervention
Intervention Characteristics		
Basic product design	Stability	Stable under normal conditions; loss of binding capacity (primary mechanism to reduce aflatoxin bioavailability) if heated ≥ 200° C over 30 minutes (372)
	Standardization	Needed for human consumption purposes, to ensure reliable dose whether in capsule form or blended in meal
	Safety profile	No significant changes in hematology, liver, kidney function, vitamin A and E levels, and mineral levels. Mild gastrointestinal symptoms have been observed. Sterilization and standardization necessary.
	Ease of storage / transport	No special requirements for storage. Transportation is needed from other parts of world where clays have shown aflatoxin-binding properties and can be sterilized and standardized.
Supplies	Need for regular supplies	A regular supply is needed in aflatoxin-vulnerable regions, because of daily consumption requirements
Equipment	High-technology equipment and infrastructure needed	If imported, no local high-technology equipment is needed. If produced locally, sophisticated manufacturing and packaging equipment is needed.
Delivery characteristics		
Facilities	Retail sector and outreach services	Depending on de livery method (capsules, blended into meal, etc.), can be purchased or distributed in food markets or local health centers
Human resources	Skill level required for service provision	Staffs are needed to distribute NS in the appropriate manner to the general public (e.g., blending the product into meal, selling or providing caplets). If production is done locally, trained scientists are required for manufacture and maintenance of the product.

Table 7-4 (continued)

Category	Criteria	Intervention
Government capacity requirements		
Regulation/ legislation	Need for regulation	May be subject to food additive regulations in target nations
	Need for monitoring and enforcement	Monitoring needed to prevent potential counterfeiting / inappropriate health claims of untested clay
Management systems	Need for sophisticated management systems	Need for government financing and management to subsidize NS if it is incorporated in major food products, or if distributed for free in capsule form. It is also necessary to manage potential risks of counterfeiting and compliance (Gilbert 2008).
Collaborative action	Collaborative efforts within government sectors and between government and other groups	Depending on NS's delivery mechanism, coordination is needed between agricultural, health, pharmaceutical, and food-related governmental sectors. Community volunteers can help government to monitor inappropriate use or the presence of counterfeiting. Because this intervention requires continuous action (monetary support), funding from international organizations may be crucial.
Usage characteristics		
Ease of usage	Need for information/education	In aflatoxin-vulnerable areas, education is needed on when, why, and how often to consume NS. May be difficult for individuals to remember or to desire to take NS capsules with each meal, so alternative delivery mechanisms should be considered (e.g., blending NS into maize or groundnut meal).
Pre-existing demand	Need for promotion	Geophagy is common in certain parts of world; however, there is a need to promote NS specifically as distinguished from common clays, and why aflatoxin is an important risk to control.

Table 7-4 (continued)

Category	Criteria	Intervention
Black-market risk	Need to prevent resale/counterfeiting	Potential risk of counterfeiting with common clays that do not adsorb aflatoxin in the GI tract

7.4.3.1 NovaSil: Basic characteristics.

NS is one of only several types of clays that can properly adsorb aflatoxin in the GI tract; hence, it is important to distinguish NS in any public education effort, to prevent the belief that any clay would have the same property. NS is stable under normal conditions of temperature and moisture; it loses aflatoxin-binding capacity if heated to over 200°C for over 30 minutes (372). Standardization is important, whether NS is administered in capsules or blended into maize or groundnut meals, to ensure effective and safe doses for humans. Although mild GI symptoms were reported in an initial human trial, Phase I (72) and Phase II (73) clinical trials confirm the safety of NS for use in human food, and provide assurance that it does not bind and result in elimination of nutrients such as vitamins A and E. Indeed, NS has a notable preference and capacity for aflatoxin (70).

A regular supply of NS would be needed in aflatoxin-vulnerable regions, because of daily consumption requirements; i.e., NS should be consumed whenever aflatoxin is present at potentially risky doses in food. Because not every African geographic region has types of clays necessary to bind aflatoxin (70), NS (or other adsorbent clays) must be imported. Hence, it is not necessary to set up the extensive high-technology equipment and infrastructure needed to produce and maintain NS throughout Africa. However, because NS must be supplied from elsewhere on a regular basis, transportation and delivery costs may be high.

7.4.3.2 NovaSil: Delivery characteristics.

Depending on the delivery method to consumers (capsules, blended into meal, or other options), NS can be purchased or distributed in food markets or local health centers. If any part of the production chain is carried out locally (including blending the clay into the meal), trained personnel are required.

If NS must be imported, as described above, transportation and delivery issues to at-risk populations are among the top priorities that need to be planned in advance. To whom, and how, will the clay be delivered, and what is the anticipated cost? Is this a universal coverage intervention? If not, which populations are the target groups? Will this intervention be used every day for an extended period of time, or only occasionally, when high levels of aflatoxin are detected in food crops? All these issues must be resolved to understand and budget for demands on delivery mechanisms.

7.4.3.3 NovaSil: Government capacity requirements.

NS may be subject to regulations governing food additives in target nations. National and local governments, in collaboration with outside partners, need to make a financial investment for the initial subsidy of NS, as many of the most aflatoxin-vulnerable populations do not have sufficient funds to purchase quantities necessary to reduce risk through NS consumption on a regular basis. There is also a need for government-funded inspection and monitoring to prevent potential counterfeiting of NS products; i.e., producing and marketing clays that do not bind aflatoxin and may indeed cause adverse health effects. Depending on the delivery mechanism of NS, coordination is needed among agricultural, health, pharmaceutical, and food-related government sectors.

7.4.3.4 NovaSil: Usage characteristics.

When considering who would use NS, and under what conditions, it is important to consider likelihood of adherence to a demanding regimen. For optimal effectiveness, consumers should take NS at every meal in which aflatoxin-contaminated foodstuffs (such as maize or groundnuts) were present. However, it is impossible under most circumstances for consumers to know whether their foods have high aflatoxin levels, so taking NS with *every* meal, regardless of aflatoxin exposure, is a possible recommended regimen.

If NS were administered in capsule form, many people would likely object to the idea of taking a capsule with every meal for extended periods of time, especially if they do not understand or appreciate aflatoxin-related health risks. Blending NS into maize and/or

groundnut meal eliminates the problem on adherence issue and has the advantage of including the product only with foodstuffs (maize and groundnuts) that could have high aflatoxin levels.

As to whether the product itself would be accepted culturally: Geophagy, the practice of consuming clay(s), is widely accepted in many parts of Africa, as well as in several other parts of the world, such as China (70). Certain African populations consume clay for several purposes, such as detoxifying dietary toxins, treating GI symptoms, and neuropsychological comfort (373). Even though promotion of NS clay as a dietary prevention is unlikely to be difficult from a cultural standpoint, promoting the right clay is important. Public educational efforts are necessary to explain the benefits of NS-enriched meal (or NS capsules; for example, in the case of emergencies), and to direct consumers toward using the right product.

7.4.3.5 NovaSil: Economic issues.

The cost of the product itself is less than one dollar per year per person for 3-gram estimated daily dose (Dr. Timothy Phillips, personal communication). Even such a low cost, however, may be unaffordable on a daily basis in certain parts of the world, where poverty is rampant and aflatoxin is a significant problem. In all likelihood, governments in collaboration with external funding agencies would need to provide the resources to deliver NS to populations in need. Indeed, this low product cost may be insignificant compared with the higher cost of transporting the material from another part of the world. NS proves most cost-effective when other methods – preharvest and postharvest – fail to prevent dangerously high levels of aflatoxin from entering the food supply. More economic research is needed on whether it is more cost-effective to only supply NS during "emergency" situations, or whether NS should become a semi-regular part of diets in certain regions of the world.

7.4.4 Hepatitis B vaccination: Technical feasibility

Hepatitis B is an infectious disease that affects the liver. The hepatitis B virus (HBV) can cause acute illness, characterized by GI symptoms, tiredness, jaundice, and muscle and joint pain. In about 10% of cases, HBV can also cause chronic infection, which can result in liver cancer, cirrhosis, and death. HBV is spread through contact with body fluids of an infected person. In

LDCs, individuals are most commonly infected with HBV through maternal-to-child transmission. HBV is also transmitted through contact with body fluids through breaks in the skin, contact with objects that have body fluids on them, unprotected sex with infected individuals, and needle sharing (374).

A regular practice now in the US and other developed nations, HBV vaccination in children is still rare in many parts of the world. Vaccinating children against HBV has been shown, over the last three decades, to significantly decrease HBV infection in several regions including Europe (91, 92), Taiwan (93), and Thailand (94). This vaccine has already had, and will continue to have, significant impacts on liver cancer incidence, particularly in Africa and East Asia.

Though the HBV vaccine itself does not affect actual aflatoxin levels in diets, it reduces aflatoxin-induced HCC by lowering HBV risk, thereby preventing the synergistic impact of HBV and aflatoxin in inducing liver cancer. For individuals who are chronically infected with HBV (common in China and Africa), aflatoxin consumption raises by up to thirty-fold the risk of liver cancer compared with either exposure alone (33). Hence, lowering the risk of chronic HBV infection through HBV vaccination could reduce by 30 times the risk of aflatoxin-induced liver cancer, and may also play some role in reducing aflatoxin-induced cirrhosis (357). However, the vaccine may not prevent other adverse effects caused by aflatoxin (e.g., immunomodulatory effects). The feasibility of including the HBV vaccine as an aflatoxin risk-reduction intervention is summarized in table 7-5.

Table 7-5. Technical feasibility characteristics of Hepatitis B vaccination for aflatoxin risk reduction in Africa

Category	Criteria	Intervention
Intervention Characteristics		
Basic product design	Stability	Vaccine should be stored between 2°C to 8°C (refrigerated, not frozen) (Drugs.com 2009)
	Standardization	Necessary to ensure that all vaccine doses are the same for a given target group, and safe for that group
	Safety profile	This vaccine is very safe. The most common side effect is pain at the site of injection. There is no clear association to other serious side effects (NFID 2009). However, it is crucial that needles be kept sterilized and that vaccines be kept refrigerated.
	Ease of storage and transport	Vaccines require cold storage (See above; applies to transportation conditions as well)
	Need for regular supplies	To reduce HBV prevalence, multi-generation vaccination is needed. Therefore regular supply of vaccine is required
	High-technology equipment and infrastructure needed	Cold storage is necessary to preserve the vaccines, which can be a challenge in areas without electricity. Existing infrastructures in hospitals and other health centers can facilitate vaccination.
	Number of different types of equipments	Temperature controlled chambers/containers Needle syringe Antigen-antibody titer check

Table 7-5 (continued)

Category	Criteria	Intervention
Delivery characteristics		
Facilities	Retail sector	Vaccines must be provided by a reliable source to ensure efficacy, cleanliness, and proper dosage
	Outreach services	Mobile vaccination services (door-to-door) may be possible and desirable in certain communities
	First level care	Community education on HBV's health effects and how to prevent infection is desirable
	Hospital care	Clinics can provide vaccination to infants and previously unvaccinated, uninfected people
Human resources	Skill level required for service provision	Nurses, medical assistants or other trained personnel to administer vaccines
	Skill level required for staff supervision	Medical staff required
	Intensity of professional services (frequency/duration)	Regular service is required to supply vaccines to health care facilities
	Management and planning requirements	Because this vaccine is not locally manufactured in most of the high-HBV-prevalent countries, planning and management of vaccine inventories and funding are two requirements. Planning should also cover how the vaccine reaches the target population, evaluation, and up-scaling of the program.
Communication and transport	Delivery dependence on communication and transport infrastructure	Cold storage needed. To reach large proportions of the target population, it is important to distribute this vaccine to every part of the country: all local clinics and health centers, if possible.
Government capacity requirements		
Regulation/ legislation	Need for regulation	No special regulation is required, but government must see HBV vaccination as priority to mobilize resources

Table 7-5 (continued)

Category	Criteria	Intervention
Management systems	Need for sophisticated management systems	Clinics and other health care centers must be connected with vaccine supply outlets, and staff should be trained to administer vaccines.
Collaborative action	Collaborative efforts within government sectors and between government and other groups	Health departments within each nation should coordinate with each other and international health organizations to provide vaccines regularly where needed. External funding is necessary, because in order to achieve widespread vaccination, continuity of the program is a vital part. Without external funding or support from external agencies, it can be difficult for poorer nations to maintain this program.
Usage characteristics		
Ease of usage	Need for information / education	Individuals must understand need for vaccine as well as where and how often to obtain it, for themselves and their children.
Pre-existing demand	Need for promotion	Vaccination has already been promoted in many African nations, but especially in rural areas, greater effort is needed to educate the public on benefits of vaccines.
Black-market risk	Need to prevent resale/counterfeiting	Low risk of resale or counterfeiting

7.4.4.1 HBV vaccine: Basic characteristics.

The HBV vaccine is made from a part of the virus, and cannot cause infection. It is usually given as a series of 3 or 4 shots, each one conferring ever-greater protection against chronic HBV infection risk. It is recommended that all children receive their first dose of HBV vaccine at birth, because of the maternal-to-child transmission risk (375, 376) and complete the vaccine

series by 6-18 months of age. Additionally, any child, adolescent, or adult who has not been previously vaccinated should receive the vaccine (374).

To maintain product stability, the vaccine should be stored between 36-46 °F (refrigerated, but not frozen; Drugs.com 2009). Generally, vaccines have been standardized during their manufacturing processes. This is necessary to ensure that all vaccine doses are the same, and at safe and effective doses, for the target population. The HBV vaccine has been used for decades safely, with low risk of significant side effects; the most common side effect is pain and swelling at the site of injection (377). However, it is crucial that needles be kept sterilized and not shared, and that the vaccine remains refrigerated at all times before use.

One main technological challenge for many parts of rural Africa lies in providing and maintaining cold storage for the vaccines (376). Cold storage is difficult where electricity is not available. Indeed, the rate of accessibility to electricity in sub-Saharan African populations was approximately 15% in 2005 (378). It is also not optimal for individuals to have to travel too far in order to receive the vaccine (e.g., to the clinic of the nearest village that does have electricity), as incentive to receive the vaccine may decrease. Other types of equipment/supplies needed include needle syringes and antigen-antibody titer checks. A regular supply of the vaccine is needed throughout populations in Africa, to vaccinate children when they are born, and to others who have not previously received the vaccine.

7.4.4.2 HBV vaccine: Delivery characteristics.

Vaccines could be delivered in at least two different general methods in Africa, to reach as much of the population as possible. One is to deliver the vaccine at existing hospitals, clinics, and other health care centers. This would be the main means by which to reach urban populations and more technologically sophisticated villages.

Another option is to deliver the vaccine through a mobile vaccination service, traveling door-to-door as necessary with cold storage in the medical vehicle: focusing at first on reaching everyone who had never been previously vaccinated, then focusing primarily on reaching newborn babies (if possible). Even if it were impossible to perfectly target the households with newborn babies, simply vaccinating the mothers in a broad vaccination outreach could dramatically reduce the risk of HBV transmission to babies. To this end, the vaccine must be kept cold but not frozen during transport. It is recommended to use frozen packs for hot-weather

conditions or refrigerated packs for cold-weather conditions during transportation. Proper insulation such as crumpled paper or buble wrap should be use to keep the vaccine from direct contact with frozen pack or shifting during transport. Moreoverm insulated container should be kept in a cool place in the vehicle if possible (379). Even with this mobile vaccination option, reaching a broad population in Africa may be difficult; as averaged transport access rate by road in sub-Saharan Africa in 2005 ranged from 60% −70% (378).

Vaccines must be provided by a reliable source to ensure efficacy, cleanliness, and proper dosage. Nurses, medical assistants, or other trained personnel can administer the vaccines. Aside from administration of the vaccine, outreach services should also be provided to educate the public on the importance of vaccination and completing the recommended regimen.

7.4.4.3 HBV vaccine: Government capacity requirements.

Initiating, preparing, and maintaining a vaccination program is an extremely complex task; and requires governmental coordination at the administrative, technical, medical, logistic, educational, financial, and political levels (376). Clinics and other health care centers must be connected with vaccine supply outlets, and staff should be trained on how to properly administer the vaccines, especially to avoid cross-contamination through needles.

External funding is almost certainly necessary in most sub-Saharan African countries, because in order to achieve widespread immunity to the disease among a population, continuity of the program is a vital part. Fortunately, the Global Advisory Group of the Global Alliance for Vaccines and Immunization (GAVI), supported by UNICEF, WHO, and the Bill and Melinda Gates Foundation, has specifically recommended that HBV vaccination be integrated into national immunization programs in all countries of the world (380). GAVI provides funds and other resources to implement HBV vaccination in nations whose gross national income is below 1000 USD per capita per annum. Hence, most sub-Saharan African nations qualify for GAVI assistance, and efforts have been made to spread HBV vaccination there since the early 1990s. Yet as of 2008, 20% of all unvaccinated children globally were in sub-Saharan Africa (376). (The only place in the world that has a greater number of HBV-unvaccinated children is India.) African nations that accepted GAVI aid now have between 50-96% coverage of infant HBV vaccination, while Nigeria, which qualifies but did not accept GAVI aid, has HBV vaccination coverage of only 27% (376).

7.4.4.4 HBV vaccine: Usage characteristics.

The goal of any vaccination program is to immunize as many individuals as possible, so as to prevent spread of the disease. It is important to promote and educate about the HBV vaccination program to encourage individuals to complete their vaccination regimen (3 boosters on average to provide near-lifetime immunity). For example, for individual boosters, coverage rate of HBV immunization in a large cohort of infants in Venda, South Africa dropped rapidly from 99% to 53% and 39% for the first dose, second dose, and third dose, respectively (381).

7.4.4.5 HBV vaccine: Economic issues.

Economic considerations for the HBV vaccine are not substantially different from those of other common vaccines in less developed countries. The vaccine itself is extremely inexpensive, considering its lifetime benefit: less than $1 per dose (127), with three doses are recommended per individual to provide up to 95% efficacy in HBV protection (112).

Even so, in order to impact as many people as possible, HBV vaccination programs in relatively poor African nations may require external funding to be initiated and/or sustained. Currently, as described above, HBV vaccination in several African countries is financially supported by GAVI. As a result of GAVI funding and other resource support, HBV vaccination among infants has increased enormously in the last decade (376). Still, there are many African nationsthat do qualify for GAVI funding but have not yet applied for it.

Economic issues surrounding HBV vaccination in Africa are largely out of the hands of individuals. Governments need to decide that HBV vaccination is a priority, and to contribute funds or apply for funds in order to reduce the burden of HBV-related disease. As populations in many of these nations are also vulnerable to high aflatoxin levels in their food, reducing HBV risk becomes an even more important problem, regarding liver diseases such as cancer and cirrhosis. This information on the synergistic risks of aflatoxin and HBV should be conveyed to governments to emphasize the importance of reducing either, or both, risk factors.

7.5 DISCUSSION

No one intervention to reduce aflatoxin risk in Africa emerges as being "most feasible" in all categories. Each has its unique benefits and drawbacks for wide-scale adoption in Africa. For example, biocontrol is highly cost-effective and reduces aflatoxin at its earliest stages. However, professional staff and training requirements may be high. The postharvest intervention package has the benefits of cultural appropriateness and adaptability to multiple different local settings, as well as low technology and equipment requirements. However, there is a high public education component for proper sorting and drying practices, and compliance requirement for health benefits. NovaSil may prove a life-saver in emergency situations when preharvest and postharvest means have failed in keeping high aflatoxin levels out of food, but long-term public adherence may be problematic. The HBV vaccine has high global-level support and needs only be administered three times in a lifetime to ensure high protection against liver-related diseases, but clinical and facility requirements are high.

As different as these interventions are from each other, certain general trends emerge from a technical feasibility study. The first is that public and governmental education on aflatoxin risk is absolutely crucial to provide economic incentives to adopt interventions. Even if an intervention to reduce aflatoxin risk is cost-effective, in terms of lives saved and quality of life improved (Wu and Khlangwiset 2009), there may still be no incentive to implement it unless health and market effects of aflatoxin are fully understood. It is worth noting that aflatoxin exposure in Ghana has been shown to be significantly correlated with farmers' knowledge of aflatoxin risk (Jolly et al. 2006), while farmers' knowledge of aflatoxin risk in Benin has been correlated with the motivation to implement aflatoxin-reduction interventions (Jolly et al. 2009).

Education must take place in at least three different levels. Government policymakers must first receive information about the burden of aflatoxin-induced disease in their nations – both in terms of health and market effects – as well as information about possible interventions, their cost-effectiveness in reducing aflatoxin, and their technical feasibility requirements. Obtaining the appropriate information will motivate them to provide the finances and other resources necessary to initiate the interventions. Also, depending on the intervention characteristics, either the farmers, or the consumers, or both these groups must receive education on why aflatoxin is a concern and how to implement the intervention in question. Finally,

international health and agricultural organizations must e informed about the extent to which aflatoxin can affect both food markets and public health. This will provide incentive to aid nations in which aflatoxin is still a significant problem in food.

The second trend is that interventions would ideally be combined in a suite to solve aflatoxin problems in LDCs. The one s analyzed in this study represent preharvest, postharvest, dietary, and clinical solutions to the problem. Each one, taken alone, could reduce a significant burden of aflatoxin risk; but potential failures in the overall system could result in gaps through which high contamination events could occur. Biocontrol would help reduce preharvest aflatoxin accumulation from the start, to ameliorate any potential problems further along the food chain. The postharvest intervention package significantly reduces aflatoxin in storage so that even food stored for longer periods of time would have greater safety. NovaSil can serve as an enterosorbent to reduce aflatoxin risk in cases where food is already highly contaminated. The HBV vaccine lowers the overall risk of specific liver diseases for which aflatoxin is a risk factor.

The third trend is that, after appropriate funds are obtained, *delivery* of the intervention to people and places in need may be the most significant challenge to implementing aflatoxin risk-reduction interventions. In three of the four case studies described in this study, the delivery would cost more than the intervention itself – in some cases, significantly more. The one exception is the postharvest intervention package, whose materials can be obtained locally. In the other cases, either the intervention must be imported, or significant effort is needed to establish the equipment and personnel necessary in various parts of the country to reach the target population.

Understanding constraints to feasibility of interventions aids scientists and policymakers to think beyond efficacy, and even beyond material costs. For interventions to succeed in less developed countries, governments, scientists, international organizations, farmers, and consumers must work collaboratively to overcome challenges in implementing the intervention – challenges in terms of human resource needs, equipment and technology and transportation requirements, financial aid, and user adoption constraints. Feasibility analyses can indicate research and development priorities in order to improve likelihood of adopting interventions that can improve public health and market outcomes.

8.0 CONCLUSIONS, LIMITATIONS, AND PUBLIC HEALTH SIGNIFICANCE

8.1 CONCLUSIONS

We have estimated that out of total liver cancer cases in Nigeria, more than 50% are HBV-related (59-62%). The liver cancer caused by aflatoxin accounts for about 8-27% of total liver cancer in Nigeria. However, there is an overlap between the liver cancers caused by the HBV infection and aflatoxin; about 7-23% of total liver cancer are the results of the synergistic effect of aflatoxin and chronic HBV infection.

Since HBV plays a major role in Nigerian liver cancer risk, and because the HBV vaccine has such high efficacy, this vaccine delivers greater benefit to prevent liver cancer cases – even those induced by aflatoxin - compared with aflatoxin-specific interventions. Aside from providing greater efficacy to prevent liver cancer, the HBV vaccine also costs the least compared with the other three interventions: biocontrol, the postharvest intervention package, and NovaSil. According to WHO guidelines to evaluate the cost-effectiveness of public health interventions, HBV vaccination is highly cost-effective. If liver cancer prevention were the only health endpoint of aflatoxin in which we are interested, the postharvest intervention package and NovaSil clay are not considered to be worthy of adoption. Biocontrol, depending on the levels of aflatoxin that would otherwise have existed, can be either cost-effective or non-cost-effective.

The results from our review on the relationship between aflatoxin exposure and growth impairment led credence to the hypothesis that aflatoxin is associated with childhood stunting in high-risk areas of the world. Though it is impossible to completely eliminate aflatoxin, it is crucial to keep levels "*low.*" High levels of aflatoxin can induce growth impairment, which results in a large public health burden.

Table 8-1. Summary of the cost and the numbers of prevented cases of aflatoxin control interventions

Intervention	Annual cost Millions USD)	Case prevented	
		Liver cancer	Childhood stunting
HBV vaccine	37.82–45.88	21,147–22,591	NA
Biocontrol	75.40	1,065–2,886	736,000–1,015,000
The postharvest intervention package	103.68–259.20	388–405	231,000–255,000
NovaSil	181.33	758–1,677	736,000–958,000

Table 8-1 summarizes the annual cost of four aflatoxin control interventions and the numbers of liver cancer cases and cases of stunting that can be prevented by each intervention. The malnourished condition in children is not associated with chronic HBV infection (382-384). Therefore, HBV vaccine does not provide benefits on preventing stunting.

Because of the complexity of the diet-disease interaction, the results should be interpreted cautiously. Aflatoxin could play a role in reducing growth performance in children who have preexisting risk factors such as borderline malnutrition, but not in children who are well nourished. However, in animal studies, aflatoxin caused growth impairment in animals fed diets similar to control animals (see chapter 5). Our study shows that if other factors related to stunting in Benin and Nigeria are similar, dietary aflatoxin exposure can induce impairment in growth performance, including stunting, which is associated with an increased risk of dying from several diseases and/or conditions, such as diarrhea, pneumonia, malaria, and measles (229).

Childhood stunting carries a greater overall burden than liver cancer for two main reasons. First the incidence of liver cancer is much lower than stunting. In general, liver cancer incidence is reported per 100,000; whereas, the incidence of stunting is per 100. S econd, compared with liver cancer, stunting occurs much earlier in life. Therefore, a life lost due to stunting-related conditions causes more than 30 DALYs.

In our study, the benefit of preventing stunting is large. Including this benefit in evaluating aflatoxin risk-reduction interventions can make the difference between their being not cost-effective to being cost-effective in terms of DALYs saved. The cost of the postharvest intervention package to prevent one healthy life loss due to liver cancer is much higher than the

three-time Nigerian GDP per capita, even when the cost is reduced to 30% of its original cost or its efficacy was much improved to completely reduce aflatoxin in groundnuts. But once the benefit of preventing stunting is included, the postharvest intervention package becomes very cost-effective and still cost-effective even when the cost is 10 times higher than its current cost. This is similar to the other two aflatoxin-specific interventions: biocontrol and NovaSil clay.

While any single intervention is very cost-effective if the benefit of preventing stunting is included and aflatoxin exposure is high enough to induce stunting, combinations of interventions are not cost-effective. Marginal cost-effectiveness assessment determines the ratio between extra benefit gained and extra cost from including an additional intervention. As a single intervention, biocontrol is able to reduce total aflatoxin exposure to be lower than the threshold of aflatoxin to induce stunting. Therefore, having an additional intervention does not increase the benefit of preventing stunting. Though the new intervention can prevent liver cancer, the benefit of preventing liver cancer in this case is very small compared to the extra cost of having one more intervention implemented. It is worthwhile to note that the results can be different if the exposure levels are extremely high, that biocontrol cannot reduce aflatoxin levels under the level "safe" for stunting. In this situation, having an additional intervention could cause a significant benefit.

However, this does not mean that the intervention which provides the greatest efficacy and the cheapest cost to prevent one bad outcome is the ideal intervention. There are other aspects of the interventions that need to be considered: basic features such as safety and standardizability, delivery characteristics, usage characteristics, and governmental support. Our technical feasibility analysis revealed that each intervention has its own advantages and disadvantages. Limited infrastructure and resources in rural areas can be the reason that the best intervention cannot be practiced. For example, the HBV vaccine–the best intervention in our efficacy and cost-effectiveness studies–requires a cold chain to ensure that the HBV vaccines are kept between 2°C–8°C and a sufficient number of public health personnel are available to perform the vaccination. This may be infeasible in many rural communities worldwide. Moreover, some religious and personal beliefs may prevent babies from being vaccinated.

8.2 LIMITATIONS

The first limitation in our studies is the absence of the data of aflatoxin levels in maize and groundnuts stored in Nigerian households. Since the products sold in markets are often stored for shorter periods compared with maize and groundnuts stored in growers' household, aflatoxin levels in household goods would likely have higher levels of aflatoxin.

Another limitation in our study is caused by the lack of information on the kinetics of aflatoxin in children. The conversion factor (37) used in this study to convert the levels of dietary aflatoxin exposure to the levels of AF-alb adducts was derived from adult subjects. However, the levels of albumin in children and adults are not much different, 34-42 mg/ml and 37-56 mg/ml for infants age 1-3 years old and adolescence age 7-19 years old, respectively (385). Moreover, it was estimated that only 1-3% of aflatoxin is bound with albumin (386). Therefore, the effect from using an adult conversion factor to calculate children AF-alb adducts should not be significant.

Currently, epidemiological studies on aflatoxin and growth impairment are limited to West Africa, East Africa, and the Middle East. The one study that showed a dose-response relationship between aflatoxin exposure and growth impairment was conducted in Benin and Togo. Because of the complexity between diet and disease, our data on stunting may not be able to generalize to other populations whose diet or preexisting factors are much different than the studied populations in Benin and Togo.

8.3 PUBLIC HEALTH SIGNIFICANCE

Public health policy makers can use our data in this study to help them appropriately allocate limited resources, such as human and monetary resources, to reduce public health burdens of liver cancer and stunting caused by aflatoxin. The data from this study would highlight the importance and the knowledge gaps yet to be filled regarding aflatoxin toxicity in children. We encourage researchers and grant providers to pay more attention to the toxicity of aflatoxin in this sensitive population. In general, the metabolic function, which is important for aflatoxin to

exert their carcinogenicity in children, is less effective compared with adults; but per kilogram bodyweight, children consume a much larger amount of food than adults. Therefore, children are considered to be a sensitive population. Importantly, if aflatoxin does not need to undergo metabolic pathways to cause impairment in growth performance, reduced metabolic function in children compared with adults put children in a dangerous situation. Finally, intervention developers can use our data to justify and improve their interventions. The postharvest intervention package can be modified to be more appropriate to apply to other food crops, such as maize, which is consumed in a much larger amount than groundnuts and plays a bigger role on aflatoxin exposure.

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