

Review Article

Integrated Biological and Chemical Strategies for Mitigating Aflatoxin Contamination Across the Food Chain

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Article Info

Article history:

Received 23 -1-2026

Received in revised form 21-4-2026

Accepted 1-6-2026

Available online 30 -6 - 2026

Keywords: Mycotoxin , Aflatoxin B1 ,Food safety , food chain

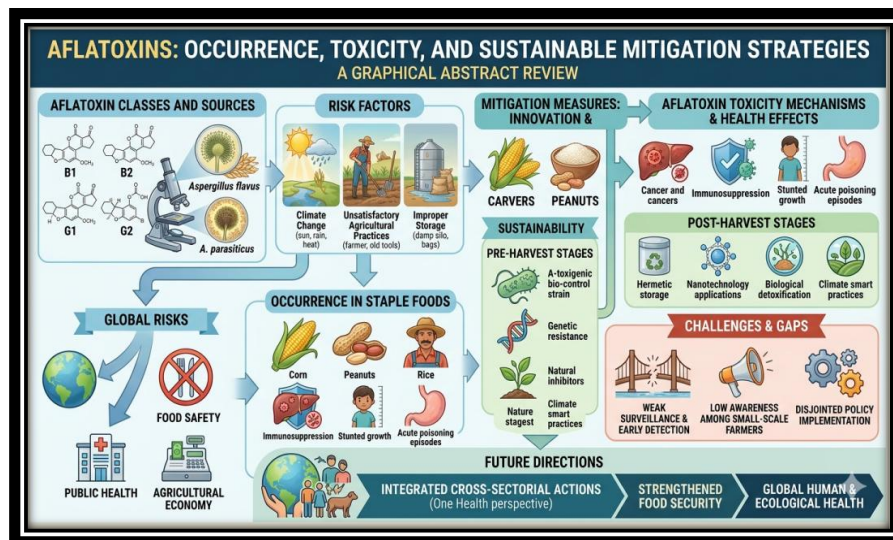
Abstract

Aflatoxins are a number of dangerous chemicals (toxins) that also cause cancer (carcinogenic) are produced by two different types of fungi *Aspergillus flavus* and *Aspergillus parasiticus*. They are a worldwide concern for food supply, agricultural, and the public health economy, especially in tropical and sub-tropical regions around the world. The focus of this paper is to bring together recent research on types of aflatoxins, where they come from, and how they are harmful (affect people negatively) to humans. Research on the food supply due to Climate Change, improper farming techniques, and improper food storage methods has resulted in an increase in the risk of aflatoxin contamination in foods worldwide. Long-term exposure to aflatoxins has been linked to causing liver cancer , weakening the immune system, stunting children's growth, and acute (sudden) poisoning. This notice will also discuss the new, sustainable ways to mitigate/prevent aflatoxin occurrences before or after harvest, such as using safe strains of bacteria/fungi to control fungus (toxigenic microbial biocontrol strains), having plants that are genetically resistant to fungi, using natural substances to prevent fungus (natural inhibitors), using new methods of storing food (hermetic storage technologies), using small particles for food safety (nanotechnology), detoxifying foods biologically (biological detoxification), and using agricultural methods that do not contribute to climate change (climate smart practices). Notwithstanding these advancements, there continue to be serious obstacles with respect to insufficient or nonexistent reporting infrastructure for surveillance/early identification systems; minimal understanding/education among small-holder farmers about this issue; and fragmented policymaking processes. The review concludes with a call for greater cooperation among various sectors, using an integrated One Health approach, to reduce the risks associated with aflatoxins and improve food security while safeguarding the health of both people and the environment globally.

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Peer review under responsibility of Iraqi Academic Scientific Journal and University of Kerbala.

Graphic abstract



Introduction

The complexity of agri-food systems is evident in both their multi-dimensional nature as well as the way they exist as a mixture of several different sectors working together to create synergy through their dynamic interrelationships of the many factors contributing to the production, consumption, trading and distribution of food. Sustainable food systems must also address all of the environmental, economic, social, and health considerations related to providing food sources which are of good quality, nutritious value, safe and secure for future generations (1). The realization of the vision requires a systems transformation of the way food is produced, processed, consumed and distributed; and the need for policies and strategies which assist with creating resilient and equitable agri-food systems(2).

Mycotoxin contamination, along with many microorganisms, is one of the major barriers preventing food safety and food security. The complex contemporary food supply chain involves many participants and processes by which food moves from production, for example, processing, storing, and transporting. At each stage of this process there is a potential risk of food being contaminated (2,3). One of the major threats to the developing world, particularly

in rural areas of developing countries, is the quality and safety of food due to mycotoxins. Mycotoxins are low molecular weight (300–700 Da), toxic secondary metabolites produced by various molds including *Aspergillus*, *Fusarium*, *Penicillium*, and *Alternaria* (4,5). Mycotoxin contamination can occur at any point from "field to fork," resulting in large amounts of food being wasted, along with significant social and economic costs to the entire food-production system (4,6).

Mycotoxins are known to be harmful substances produced by many different types of fungi; however, they can also be produced artificially. Aflatoxins (AFs) are among the most toxic and harmful types of mycotoxins; AFs can be found in feed and food products, and thus pose a potential risk to the health of humans or domestic animals due to the occurrence of a number of pathological processes (whether they be cellular or physiological in nature) as a result of exposure to AFs. The mutagenic, teratogenic, genotoxic and carcinogenic properties associated with long-term exposure to AFs have been documented during studies on their effects either in vivo or in vitro. Humans and animals exposed to AFs have shown various organ dysfunctions including dysfunction of the liver, kidneys, gastrointestina-

tract, reproductive organs and immune system (7). Also, human development from fertilization through to the early part of life or into the embryonic stage may be affected by aflatoxins, resulting in developmental delay (retardation), and neurodevelopmental delay, and/or compromised immune system function (8). It is thought that oxidative stress likely plays a significant role in triggering the induction of this AF gene cluster; however, the environmental production of mycotoxins by *Aspergillus* organisms is highly intricate. The wide-ranging interactions between AF-producing fungi, plant hosts, and the soil micro biota and macrobiotic require additional investigation to increase the efficiency of developing and refining methods for preventing AFs (9).

Examples of preharvest bio control agents that have demonstrated the ability to inhibit the formation of aflatoxin producing molds include(a) numerous approaches to controlling the aflatoxin toxicities will use bio-control approaches that rely on the biological attributes or bio pesticide properties of various bacteria, yeast, and fungi (as well as their metabolites)and (b) these types of biological agent/preharvest bio control systems have been used to commercially apply and successfully utilize numerous combination preharvest bio control strategies(10).

Aflatoxins (AFs) contaminate the food and feed chain at every level, starting from the field to when the feed and food are consumed, and in many different kinds of plant products such as cereals, nuts, spices and fruits (11). After ingestion, AFs are absorbed from the intestine and transported by the blood to areas of the body in both animals and humans, where they may also be chemically transformed into additional hazardous substances. Aflatoxin M1 (AFM1) and other undesirable substances will ultimately be excreted by the animal/human,

and can even be found in milk from the excretion (12). Due to milk being excreted from animals, Extensive reports of indirect contamination of milk and dairy products with dangerous levels of AFM1 have been found in the literature, while there have also been reports of direct contamination of milk and dairy products via the presence of molds and their mycotoxins (13). According to research, it is possible to detect aflatoxins (AFs) in human breast milk after they have been converted into aflatoxin M1 (AFM1). This poses a serious threat to young, vulnerable infants who are breast-fed (14). Aflatoxins (AFs) also cause embryonic developmental defects through the placenta and fetal organs while in utero (15).

Aflatoxin B1 (AFB1) is the most common and toxic aflatoxin. It is considered to be highly genotoxic and carcinogenic , Overall aflatoxin production and contamination can be increased due to extreme temperatures or high humidity levels during storage or after harvesting (16). Climate change is increasing the risk of exposure to aflatoxins. Climate change influences fungi's ecology and widens the geographic range of aflatoxin-producing species (17,18). The human health impacts that are associated with the ingestion of foods and feedstuffs that are contaminated with aflatoxins include serious gastrointestinal and liver-related problems, toxicity associated with the immune system, and cancer, especially Hepatocellular (liver) cancer (HCC). Aflatoxin M1 (AFM1) is significantly less toxic than AFB1 yet it continues to pose a risk to human health (4). Several countries and international organizations have established maximum residue limits (MRLs) to control the amount of aflatoxin allowed in food and feed. For example, the European Union has implemented MRLs for aflatoxin and has

put in place Key Performance Indicators (KPIs) to help member states reach their goals related to aflatoxins and ultimately the achievement of the Sustainable Development Goals (SDGs). A variety of interconnected factors affect these systems, including limits set by the U.S. and the WFP for AFB1, which is set at 2µg/kg and 4µg/kg of total AFs in addition there are limits of 20µg/kg and 10 µg/kg respectively; however, these figures account for only one component of total aflatoxin contamination(19).

In contemporary literature, there are roughly 24 aflatoxigenic species classified within *Aspergillus*, most of which can be classified into three taxonomic sections, namely *Nidulantes*, *Flavi*, and *Ochraceorosei* (20,21). Among these species, *A. flavus* and *A. parasiticus* produce the most aflatoxin and pose a significant threat to food safety and public health globally (22). Another factor that increases susceptibility of cereals to aflatoxin contamination is that maize, rice, sorghum, and millet have a high potential for being contaminated by aflatoxins when grown in warm, humid climates (23).

Contamination of aflatoxins (AFs) may occur throughout the first stage of production, including into foods of animal origin. Animals will accumulate AFs from feed into their tissues, such as the liver and excrete metabolites of the AF such as aflatoxin M1 in milk (24). The mold can sometimes grow directly onto meat products or can be the result of secondary contamination from processing and spoilage of the product. This path emphasizes how important holistic monitoring and control strategies of crop and animal feed and products is to protect the entire food system (25).

AFB1 is classified into Group 1 of the IARC, as an agent considered to be carcinogenic and toxicologically classified as B1 > G1 > B2 > G2; therefore, exposure to AFB1 through ingestion of contaminated food has been highly associated with HCC and appears most prevalent within countries that do not have any measures in place to provide effective food storage and regulation (26,27).

AFB1's hepatotoxicity is mainly the result of its metabolic transformation in the liver. AFB1's primary metabolic transformation is via cytochrome P450 isoenzymes, namely CYP3A4 and CYP1A2, which further convert AFB1 to the highly reactive metabolite (intermediate) AFB1-8,9-epoxide. The epoxide of AFB1 reacts covalently with DNA, forming mutagenic adducts (mutations) primarily at codon 249 of the TP53 tumor suppressor gene; codon 249 mutations are highly prevalent in HCC patients exposed to AF. In addition to its genotoxic effects, AFB1 causes liver toxicity by generating oxidative stress since reactive oxygen species (ROS) from oxidative stress result in degradation of cellular lipids, proteins, and nucleic acids (28). The disruption of cellular activities due to oxidative stress leads to apoptosis. AFB1 furthermore produces mitochondrial dysfunction, thus decreasing cellular energy production (ATP synthesis) and activating the intrinsic apoptosis signaling pathways (29).

AFB1 has immunotoxic properties that compromise both innate and acquired immunities. Research has shown that mycotoxins (AFB1, in specific) affect the immune system, including decreased lymphocyte proliferation, altered cytokine production, and increased susceptibility to infections after exposure. Recent studies indicate that AFB1 is also neurotoxic and can cross the blood-brain barrier, resulting in

oxidative stress leading to neuronal apoptosis and metabolic changes found on behavior/ cognition in animals(30).

Data is more readily available on cases of acute aflatoxicosis because there is a higher risk of mortality associated with this type of illness. One well-recorded incident occurred in Kenya in 2004, when eating large quantities of heavily contaminated maize resulted in at least 125 deaths from acute AFB1 ingestion. (31).

Economic Impact of AF Contamination and Food Security, Health

Aflatoxins contaminate food supplies and threaten food safety, economic development and human health in agricultural-dependent low and middle income countries (32). Aflatoxins reduce crop yields, influence commodity prices, and reduce income for farmers - resulting in the prevalence of food insecurity. In addition to diminishing agricultural productivity, aflatoxin exposure is associated with significant health outcomes, such as hepatocellular carcinoma , immune suppression and childhood stunting. Aflatoxin exposure also leads to increased economic costs associated with health care services spent, lost productivity from illness, and decreased trade through the loss of previous market access (33). The growing body of peer reviewed literature clearly demonstrates that the various impacts and outcomes from aflatoxins on, public health food systems ,and economic sectors require a collaborative response. To prevent aflatoxin exposure and its associated public health consequences, a multi-faceted approach is needed that includes biological strategies, appropriate agricultural practices prior to harvest, appropriate agricultural practices after harvest and credible detoxification

processes, as well as sound, evidence-based legislative measures. (34).

Food Security Implications

AF contamination also particularly affects staple crops, including maize, groundnut, and cereals—important elements of the diets in several African countries (35). The food security condition of being able to access adequate, safe and nutritious foods is facing many challenges from AFs. These mycotoxins undermine all four components of food security: availability, access, utilization and stability (19,33). It results in pre- and post-harvest Losses, and lower yield which in turn lowers food availability. If the contaminated crops are not accepted in the markets or commanded a lower price, farmers' income reduced and they could afford less food. Quality of the screw crushed foods may also reduce and thereby reduces food diversity, leading to malnutrition. Furthermore, supply chain instability is added to when crops are lost due to contamination or diverted away from food uses (16).

Sustainable and Innovative Mitigation Strategies of AF Contamination

With the food systems in a global crisis due to climate change and globalization, there is an urgent need to develop sustainable and novel approaches of controlling aflatoxin contamination (36). Comprehensive, multi-pronged measures are necessary— combined prevention, detection and response measures throughout the entire agriculture value chain (37). Effective measures to combat this threat include the integration of bio control strategies, environmental focused and technological advancements (i.e. biotechnologies, technologies based on nanomaterial's, predictive

modeling), wholesome educational aspects and active community involvement in such program (38). This holistic approach facilitates to reduce aflatoxin contamination, enhance sustainable food system and agricultural practices for the sustainable future.

Sustainable and Innovative Pre-Harvest Strategies

The application of non-toxicogenic *A. flavus* in toxigenic toxin use (produced for instance as a commercial product, such as Aflasafe/strain AF36) have consistently reduced maize and groundnut field trial aflatoxin levels by 70% to 99% using competitive exclusion of toxigenic strains (39). GAPs combined with GRDs can significantly reduce fungal contamination and subsequent aflatoxin production in fields (40). However, limitations include cost and access for small-holder farmers, incomplete knowledge of health risks involved; possible ecological displacement in fungal populations and the long-term persistence of introductions or adverse impact on *Aspergillus* community (18,41).

Genetic approaches (e.g., gene silencing, host-induced RNA interference) have shown in field and greenhouse studies promising reductions in aflatoxin levels, are sustainable over the longer term by not requiring chemical input application for control and can be incorporated into wider food security programs (40,42). Their disadvantages include long generations for resource-demanding breeding cycles, regulatory constraints and public opposition to GMOs, the variable efficacy under field or even genotype conditions; high cost and lack of stability in bioactive formulations as well as no record of systemic antifungal activity beyond controlled clamp assay.(18,43).

Bio control With A toxigenic Strains

The biological pest control method provides a safe and environmentally sound alternative to chemical pesticides, which have the potential to be harmful to non-target animals, humans and the environment. One of the primary methods of biological pest control are using (43,44). However, there are some disadvantages of using toxigenic strains .The degree to which microbial antagonists are able to effectively combat the mycotoxin AFB1 in corn (*Zea mays*) and other crops is to a great extent dependent upon a variety of environmental conditions (e.g., temperature, humidity, and drought stress) at the time of the particular application (35; 36). The selection of the correct microbial antagonist strain requires a sound understanding of the ecological systems of the target crop and its related microflora, and success depends greatly upon the antagonistic ability of the particular microbial strains having a good probability of being competitive within the target environment, i.e., a highly effective strain within one geographic region may not be nearly as effective in another region due to differences in the genetic diversity of the *A. flavus* population(45); the potential for some non-toxicogenic isolates to produce other toxic metabolites such as cyclopiazonic acid makes the careful evaluation of their safety extremely important too. Furthermore, it is necessary to conduct extensive field testing of newly developed microbial candidate strains with demonstrated high efficacy under field conditions prior to their general release in order to provide assurance to growers of a relatively high consistency in the rates of return from their use(46,47).

Microbial and enzymatic detoxification are more environmentally sound options for decontaminating substances than using chemical or physical options. For instance, *Bacillus* species have shown great potential to degrade Aflatoxin B1 (AFB1). *B. Velezensis* DY3108 has been reported to achieve a maximum degradation rate of 91.5% over 96 hours at 30°C (48) while *B. amyloliquefaciens* WF2020 achieved over 80% degradation rate within 72 hours of culture. Other examples of bacillus-derived enzymes that are effective biocatalysts in the conversion of AFB1 into non-toxic forms such as AFQ1 include lactases and particularly the CotA enzyme produced by *B. subtilis* (49). Furthermore, when the heterologously expressed (AF-) degrading enzymes from these organisms were transferred into *Pichia pastoris*, 78.9% degradation of AFB1 was achieved also after 24 hours of incubation. Similarly, *Bacillus spp.* have exhibited comparable biodegradative capabilities (50).

Post-harvest AF-Prevention of Aflatoxins (AF) relates to keeping harvest in optimal storage conditions. Using hermetic technologies such as PICS bags will help to keep moisture and oxygen from entering the grain and inhibit the growth of fungi during storage (51). A randomised, controlled trial was carried out in Zimbabwe and found that grains stored using PICS bags contained significantly lower levels than grain stored using traditional methods; therefore, using hermetic storage produced higher quality grains and increased market value for the grain, resulting in more profit for farmers (52).

Due to concerns regarding synthetic chemical control's toxicity and environmental toxicity, (53). The existing chemical options for AFS reduction (ozonation, ad-

sorption with various chemical agents, various chemical agents) typically consist of various chemicals, with some organic/inorganic acids (i.e., citric acid, tartaric acid, lactic acid, propionic acid; hydrochloric acid) are generally, but not universally, more effective than acetic acid, succinic acid, ascorbic acid or formic acid, all of which are only partially effective. Citric acid has been shown to reduce the amount of AFB1 in duckling feed by 86-92% compared to lactic acid (67% reduction) (54). Sodium bisulfite has been shown to effectively degrade AFB1 (AFB1). Sodium bisulfite is a powerful reducing agent that helps create AFB1-sulfonate derivatives. This method can be further enhanced using co-thermal treatment (i.e., heating) and by adding ozone and hydrogen peroxide. This method allowed up to 28% reduction of mycotoxins from AFB1-contaminated dried figs at an ambient temperature of 25 °C. After sodium bisulfite treatment, a combined treatment of hydrogen peroxide at 0.2%, was done for 10 minutes, and AFB1 reduction of 48 to 68% was achieved with H₂O₂ and heat (45 °C for 1 hour or 65 °C for 1 hour). The ammonium persulfate oxidant was also used to reduce the AFB1 content by 31% to 51%. AFB1 is greatly reduced in an alkaline system(55).

Certain redox-sensitive enzymes may also represent new methodologies for treating Aflatoxins (AF) through chemistry (for example). a bacterium-derived ethylene oxide (Rh_DypB), an enzyme has been reported to degrade AFB1 (Aflatoxin B1), in vitro (56). The combined use of 0.1 U/mL of the enzyme with 0.1 mM hydrogen peroxide resulted in an aggregated bioconversion of 116% in 96 hours, suggesting that such enzymes can arrest mycotoxin proliferation in animal feeds. In addition, ozone is a potential chemical weapon against AF;

when ozone interacts with products stored in grain , it may prevent the formation of mycotoxins through its high chemical reactivity (57). For total elimination of AF in cereal grains, flour or product, soybeans, peanuts and even meats, ozone was as an anti-mycotoxin agent (58). There are several different types of chemical compounds that can be used to treat AFM1 in milk. For example, some companies have developed technologies that use different forms of ammoniation, acidification, oxidation, and reduction to remove AFM1 from milk products. The use of ammoniation can potentially cause the removal of up to 90% of the AFM1 from the product(59).

Ammonia can be used in a concentration of between 0.5 and 2.0 percent to reduce levels of AFM1 in milk when it is used at pressure from 45 to 50 psi with moisture content of between 12 and 16 percent for less than one hour. Chlorine dioxide gas is also an effective method for removing AFB1 residue from maize (60).

Some examples of natural inhibitors that can be obtained from plant extracts, microbial metabolites and food grade components include natural antifungal compounds found in the essential oils and phytochemicals in plant extracts, and those found in certain ingredients used in food (61). Many of these compounds have strong antifungal properties and anti-storage aflatoxigenic properties, including bioactive substances found in the essential oils of clove, thyme, cinnamon and oregano that are eugenol, thymol and cinnamaldehyde that can help to inhibit the growth of aflatoxins produced by aflatoxigenic fungi.

Fungal cell membrane structure, and some substances can suppress toxin biosynthesis. These oils improved effective in decreasing AFB1 production (62).

In addition, some phenolic substances including flavonoids, phenolic acids and tannins in herbs like turmeric and garlic have been shown to affect the metabolic activity of fungi and inhibit the expression of many aflatoxin (AF)-related genes; extracts of ginger and moringa have also been shown to help reduce levels of AFs in stored grains, providing additional evidence for the positive effect of plant-derived materials in the context of postharvest practices (63,64).

Bentonite is a naturally-occurring adsorptive clay that is mainly made of montmorillonite. There has been an abundance of research conducted on montmorillonite's characteristics such as having a large surface area, possessing a high swelling capacity, and having a high adsorption ability, which all contribute to the enhanced ability for toxins to bind to it and thereby decrease nutrient loss (65). An example of this high efficiency is the demonstrated ability to reduce aflatoxin B1 (AFB1) by greater than 90% in the digestive tract of animals in clinical studies resulting in less available and reduced toxicity of AFB1 both in vitro and in vivo (66) over time also showed; increased use; further demonstrating the efficacy of montmorillonite in preventing the absorption of AFB1 by domestic animals and helping to eliminate AFB from the bodies of domestic animals that were treated with montmorillonite.

Evidence over the last five years have shown that adding bentonite into poultry, beef and pork diets can protect against the impact of both aflatoxin-induced liver damage; improve feed efficiency (IMR) significantly; reduce the presence of aflatoxin in milk and meat (67). By using adsorbent clays to mitigate the presence of aflatoxin this can provide a cost effective and practical way to reduce the risk of contamination

by *Aspergillus* spp, which represents one of the major challenges to agri-food systems globally, especially in tropical and subtropical countries, due to the severity of human health, food safety and economic impact consequences related to aflatoxins. (68).

Conclusions

In tropical regions of sub-Saharan Africa and South-East Asia, the burden placed upon society from Aflatoxin contamination will continue to be a major public health challenge due to the combined effects of the environmental conditions there, limited infrastructure for preventing further contamination and socio-economic disparities that impact the population's risk from chronic exposures to Aflatoxins. This narrative review has combined current knowledge on the prevalence, health effects, risk factors and remedial actions taken to date in order to reduce the impact of Aflatoxin exposure.

Bio control methods - specifically, strain selection for ataxigenic cultivars - have proven to be successful at reducing AF amplification (up to 100% in ideal conditions). Additionally, new detoxification processes such as nanotechnology-based techniques and ozone-based decontamination provide alternatives to traditional chemical and physical decontamination approaches. The methodologies tested were not widely adopted in part due to cost, technical complexity, and lack of local validation. Despite scientific and technological advancements, many basic deficiencies still exist when it comes to these methods of AF removal. Issues relating to the availability of low-cost, accurate, and reliable testing, a lack of knowledge and education among farmers, and a lack of regulations among particular areas continue to complicate this issue.

Efforts for outreach education are all critical steps to constructing Aflatoxin-resilient systems. Innovative technologies, machine learning and IoT, along with predictive analytics may drastically change how we monitor and develop early warning systems, particularly when blended with an integrated data-based framework. Additionally, a One Health approach will help establish links among agriculture, the environment and public health to aflatoxin control measures, ultimately leading to a safer, sustainable and risk-mitigated food supply change. We additionally suggest that children under 15 years of age and individuals with chronic health conditions receive protection against aflatoxin exposure due to their heightened vulnerability to adverse health impacts. In order to reduce risk, municipalities should utilize the existing networks of agricultural extension agents to provide education to the community on safe food storage practices and on safe cleaning and handling of crops throughout the supply chain. To prevent outbreaks of acute aflatoxicosis, we will need a multi-sectoral (Agriculture, Trade, Health, and Industry) coordinated approach through the Ministries involved, as detailed in the recommendations. Stakeholders need to develop and implement a national human monitoring system to track aflatoxin levels in humans, which will serve as an early warning for the initiation of emergency procedures. Enforceable aflatoxin contamination legislation for all food supply chains, along with stringent zero-tolerance policies on non-compliant organizations, will be needed as well.

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