SPECIAL GUEST EDITOR SECTION

Mycotoxin Crises: Fit-for-Purpose Analytical Responses in the Developing World

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In developed market economies, control of mycotoxin exposure in the general population is achieved by legislated regulations governing maximum permitted levels. Such regulations are widely enforced to prevent outbreaks of overt mycotoxicoses. In developing countries, particularly in Africa, the situation is reversed, and individual mycotoxin exposures can be high, especially in rural communities reliant on subsistence or small-holder farming and local markets. Besides the effects of chronic mycotoxin exposure, Africa in recent years has experienced outbreaks of acute toxicity, such as aflatoxicosis. Recognizing and handling mycotoxin-induced health crises requires a range of responses, many of which rely on the provision and availability of fit-for-purpose analytical methods. Although regional laboratories may be able to provide support, rapid responses require in-field test kits reliant on antibody technologies. The future development of aptamers into test systems may be an important component of these analytical responses, as they provide important advantages in terms of stability, shelf-life, and low production costs.

In countries of the developed world, food security and safety are ensured by the availability of sufficient resources to provide quality food and to detect and prevent the exposure of populations to mycotoxins. Food safety authorities maintain surveillance activities, particularly of imported food items most likely to present a source of mycotoxin exposure. Enhanced surveillance may even be applied to food items sourced from production areas known to be problematic in this regard. These measures are backed by rapid alert systems and, when necessary, product recalls along the supply chain. The generally varied diet prevalent in these countries is another potential source of protection in preventing a single contaminated item from predominating in the diet. The socioeconomic status of the general population also results in local markets competing with respect to food quality rather than price. In developing countries, especially in Africa, many of these food safety determinants are absent or reversed. Even where mycotoxin control regulations exist, the general lack of adequate economic resources means little enforcement. This issue is exacerbated in subsistence and small-holder farming communities that are self-reliant for food supplies and not monitored for regulatory compliance (1, 2). In many cases, these communities have limited dietary variety and are over-reliant on cereals such as maize that makes them susceptible to high levels of toxin exposure. Maize is the most widely grown cereal crop in Sub-Saharan Africa, and its consumption at daily levels of 400–500 g can result in exposures above the tolerable daily intake level of mycotoxins, such as fumonisins, even at fairly modest contamination levels (3).

Of the five agriculturally important mycotoxins (aflatoxin, fumonisin, ochratoxin A, deoxynivalenol, and zearalenone), aflatoxin has received the most attention (1). Studies in African countries have helped to establish its role as a causative agent in primary liver cancer and childhood growth retardation (1, 4, 5). Apart from these, it has been postulated to play a negative role in nutrition outcomes and to suppress immune responses to bacterial challenges in exposed populations (6, 7). More recent research has indicated that this immune suppression can work synergistically with HIV, and hence impact the onset and severity of AIDS (8). Notwithstanding the poor health outcomes of chronic exposure, aflatoxins remained a much ignored public health issue (9). However, deaths in the last decade due to outbreaks of acute aflatoxin poisoning (aflatoxicosis) have galvanized greater political response. The most severe outbreak of aflatoxicosis reported in Africa to date was in rural parts of Kenya in 2004 in which, over the period of January to June, there were 317 cases reported and 125 deaths due to high aflatoxin levels in the subsistence maize-based diet (10). In 2016, reports were received of an aflatoxicosis outbreak and accompanying deaths in Tanzania, again in areas heavily reliant on maize staple diets (11). The situation in Kenya and the realization that aflatoxin poses a major health problem in many parts of Africa has led to the establishment of the Partnership for Aflatoxin Control in Africa (PACA; 12).

Risks of Mycotoxin Exposure

Adverse health effects of mycotoxin intake may conveniently be described as chronic effects from the long-term intake of relatively low mycotoxin levels and acute poisoning due to short-term intake of very high mycotoxin levels. Conventional risk analysis of these health effects comprises risk assessment, risk management, and risk communication. Risk assessment is composed of a combination of hazard identification, hazard characterization, and exposure assessments. Of the mycotoxins known to occur in the African diet, aflatoxins,

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and aflatoxin B_1 (AFB_1) in particular, pose a major health problem due to their contamination of cereals and groundnuts. *Fusarium* toxins, particularly fumonisins, which occur widely on maize and can co-occur with aflatoxins, also pose a significant health risk. Exposure risk from both mycotoxins, either individually or jointly from cocontaminated maize or separate dietary sources, is exacerbated by high maize consumption, which is common in many African populations. Both mycotoxins have deleterious effects on liver function. Aflatoxin is a human liver carcinogen, whereas fumonisin can act as a strong cancer promoter. Coexposure of populations to and co-occurrence of these two carcinogenic mycotoxins has recently been reviewed by the Joint Food and Agriculture Organization/World Health Organization (WHO) Expert Committee on Food Additives (JECFA; 13). Although some evidence exists of additive or synergistic effects between these toxins in animal studies, there is little human data on coexposure. Nevertheless, concerns were expressed by JECFA. Although only one outbreak of human disease due to acute fumonisin exposure has been reported in India (14), aflatoxicosis has been periodically reported in Africa (10, 11) and has required action by health authorities. Analytical investigations of the aflatoxicosis outbreaks focused on determining aflatoxins in the ingested diets and local food markets (10, 15), but the co-occurrence of fumonisin and its possible cocontribution to the food-borne disease outbreak remains unknown. These two scenarios, chronic and acute exposure, require different responses from risk managers and different communication messages. For chronic exposures, long-term solutions involving agricultural, crop drying, storage, and processing improvements may be necessary, whereas acute mycotoxicoses require immediate and fast response.

In the case of aflatoxin, the resulting aflatoxicosis with its symptoms of liver damage resembling jaundice may be confused with other diseases, such as viral infections affecting the liver. Aflatoxicosis may, thus, only be part of a differential medical diagnosis that initially concentrates on infectious agents commonly encountered in the affected areas. Thus, the possibility of a food-borne liver disease may be initially overlooked. If communications to the central authority are slow, the alert of central authorities to an outbreak of mycotoxin poisoning may be delayed and a crisis initiated when large numbers of patients present at hospital with similar symptoms. In this regard, the sensitizing of rural health professionals to the possibilities of a food-borne mycotoxicosis is important.

Analytical Responses

Food Analysis

The first response to a suspected food-borne mycotoxicosis is to sample food sources from the affected households for analysis. In rural areas, this may involve long distances from the hospital, and it should be recognized that the most contaminated batch may have been consumed already. Although sampling for accurate aflatoxin analysis is known to require large sample amounts due to the extremely heterogeneous distribution of the toxin, this may not be practical in a village setting in which food security is a challenge. Consideration may also need to be given to the purpose for which the sampling and analysis is being conducted. If it is to confirm aflatoxin exposure in the patients, then that part of their store from which food was most recently taken needs to be prioritized, whereas if it is necessary to determine current ongoing exposure, as may be necessary when other villagers are still consuming from the suspect supply, then a measure is needed of the whole store. Apart from home sampling, collection of maize from village markets can give an indication of how widespread the problem has become. Given that the outbreak could be a continuing problem, mycotoxin analyses need to be prioritized. Here, the availability of laboratory facilities and resources becomes an issue, as well as the remoteness of rural areas from the requisite laboratory infrastructure. In African countries where recent aflatoxicosis has been identified, in-country laboratory expertise has existed. In general, TLC still plays an important role in aflatoxin analysis in African laboratories, although better-equipped facilities may have access to HPLC or to the measurement of direct fluorescence after immunoaffinity column cleanup. All these methods are more than adequate to identify highly contaminated food supplies in which levels could be orders of magnitude greater than those being analyzed in import control laboratories looking to maintain low regulatory maximum levels of the order $\leq$20 μg/kg. In the affected districts of Eastern Kenya, contamination levels as high as 48 000 μg/kg have been recorded (16).

Some of the problems associated with the establishment and running of mycotoxin laboratories in developing countries, including type of analytical methods and instruments, have recently been reviewed (17, 18). Although the removal of samples to distant laboratories may provide better analytical control and confidence in the results, field measurements with immediate results are an attractive option, particularly in acute situations in which it is necessary to find farmers' contaminated stores before more exposure occurs. One of the first attempts to provide this was in India with the development of a portable TLC kit in a large suitcase-sized box containing a sample grinder, domestic blender for extraction, a glass TLC tank, and solvents (19). A handheld UV lamp was used to visualize aflatoxins separated on silica plates. An LOD of 10 μg/kg was more than adequate for investigating outbreaks of aflatoxicosis and ensuring safe food in the village. An advantage of the TLC method is that it gives an estimate of aflatoxin levels, as opposed to a simple yes/no answer for some test systems that fail to indicate the magnitude of the problem. As a response to the aflatoxicosis outbreaks in Kenya in 2004 and 2005, the Centers for Disease Control and Prevention (CDC) and WHO sponsored an international workshop which noted the use in Kenya of a field screening method (dipstick) that used a simple 20 μg/kg cutoff and suggested that, although this was useful, a screening method that gave a better indication of levels would be beneficial (20). In this regard, it may be necessary, when selecting a test method or kit for emergency measurements, to also consider the measurement range of the kit, as a large range of contamination levels may be found in these situations.

Although the above TLC kit provides a portable analytical solution, it still requires operatives skilled in the use of TLC plates. TLC remains more suited to the laboratory bench than the village bench. The development of antibody-based immunological analytical techniques has provided both a rapid method and one that can be adapted to in-field work. Although these techniques were originally designed as ELISA formats, the application of antibodies to lateral-flow devices,

such as dipsticks, provides the technological base to develop suitable mycotoxin kits for work in villages. When coupled to an optical reader, the system provides semiquantitative results that can be obtained without resorting to transporting samples to distant laboratories. This concept has been practically developed by the World Food Program (WFP) in their "Blue Box" for in-field analysis under their Purchase for Progress program. Originally meant and equipped for general cereal quality testing, the addition of equipment for aflatoxin testing (mill, timer, pipet, extraction solvent, lateral-flow device, and a battery-powered optical reader) has provided a highly portable mycotoxin testing system that is not reliant on infrastructure, such as the availability of electricity or highly trained operators (21, 22). Rural farmers in Ghana have been trained by the WFP in the use of these portable devices.

Recent advances in analytical chemistry have shown that aptamers may provide viable alternative recognition probes in mycotoxin testing kits (17, 23). They are single-stranded oligonucleotides that, once identified, can be chemically synthesized at a relatively low cost. Their chemical stability means they have long shelf-lives and can endure a range of environmental conditions, such as long transport in hot, humid conditions, as may be required in areas requiring emergency monitoring. A sensitive aptamer-based dipstick based on recognition of AFB_1 and fluorescence response has been developed with a cutoff as low as 0.3 μg/kg in maize (24). Apart from optical responses, biosensors based on aptamer-recognition probes coupled with measurements of electrochemical responses also offer an alternative technology for the development of portable kits (25). A portable electrochemical biosensor has been reported to have been developed in Uganda and is based on competitive immunosensing using anti-AFB_1 antibody as the recognition probe and potentiostatic measurement for response monitoring (26).

Biomarkers

In an investigation of suspected aflatoxicosis, the food analysis described above provides circumstantial and indirect evidence for a medical diagnosis. In this regard, a suitable biomarker would provide a measure of exposure at an individual level that accounts for quantity of food consumed, level of toxin in the food, degree of toxin absorption, and, if possible, liver metabolism and activation. Such a biomarker also overcomes problems of representative food sampling, diet composition, and any effects of the food preparation technique.

In the case of aflatoxin, a number of biomarkers of exposure have been developed, generally based on in vivo metabolism of AFB_1 (27). This mycotoxin can be hydroxylated by specific cytochrome P450 enzymes to AFM_1, which is then excreted in urine, where it can be measured by suitable HPLC methods. Of more interest are the products of AFB_1 activation, AFB_1-8,9-epoxide, the active intermediate in the interaction of aflatoxin with proteins and DNA. This epoxide can bind with lysine in albumin, thus providing a serum-based biomarker, or, in its *exo*-form, with DNA to finally yield an AFB_1-N^7-guanine metabolite in urine. Both above urinary biomarkers represent relatively short-term intakes, whereas, given the long half-life of serum albumin, the serum biomarker is useful as a measure of longer-term exposure. Its measurement in serum collected from patients during the aflatoxicosis outbreak in Kenya has helped to definitively establish the cause of the food-borne disease as aflatoxin poisoning (10). Whether urinary or blood biomarkers are used, sampling requires both the ethical approval of subjects and careful adherence to protocols designed to reduce risk of exposure of health workers to infectious agents that may be present in these physiological fluids.

The serum albumin biomarker was originally measured by a specific ELISA, the results of which have been correlated successfully with those obtained by HPLC-tandem MS (MS/MS), although the levels measured by species-specific HPLC-MS/MS are lower than those measured by the general antibody response of the ELISA (28). A recent International Agency for Research on Cancer (IARC) working group on aflatoxin/fumonisin control measures noted the usefulness of the albumin biomarker for long-term exposure studies, but found that the analytical method is only available in a limited number of laboratories (29). The group concluded that it would be advantageous for the method to be more generally available. Besides its usefulness in biomonitoring, more general availability would also be of use in a rapid diagnosis of aflatoxicosis cases and in risk management responses to any aflatoxin crisis.

Whereas various aflatoxin biomarkers of exposure have been developed, similar advances for other mycotoxins have lagged behind. In terms of importance in Sub-Saharan Africa, a fumonisin biomarker would be useful in biomonitoring and epidemiological studies. Despite various attempts, the only option currently viable is the urinary measurement of fumonisin B_1 (FB_1). The drawback here is that it measures only very recent fumonisin intake and, due to the very low levels in urine, requires LC-MS/MS instrumentation with its very low LODs (30, 31).

Management Responses to Food-Borne Mycotoxicoses

As described in the introduction, negative health outcomes due to mycotoxins in general or aflatoxins in particular, can be considered as those due to chronic intakes or those associated with acute poisoning caused by very high short-term ingestion. These two scenarios require different management responses. The IARC Working Group on aflatoxin control measures addressed intervention methods including dietary diversity, genetic resistance of cultivars, biological control, postharvest measures, and chemoprevention (29). They assessed these for efficacy, readiness for implementation, and further research needs, including methods that may be suitable for acute exposure incidences. Of the methods considered, the most promising may be the use of enterosorbents, such as dioctahedral smectite clays, chlorophyll, and chlorophyllin that bind aflatoxin in the gut, preventing its absorption. However, they remain untested in outbreaks of aflatoxicosis. Currently, the main intervention method used by governments in affected areas has been one of food (maize) replacement, using supplies of maize with acceptable aflatoxin levels (32). How to handle confiscated and contaminated maize, apart from incineration or dumping, remains a problem. It is clear that in implementing a policy of maize replacement in affected rural villages, the availability of rapid testing equipment would ease the burden of this process in delineating the areas that are most in need of an uncontaminated food supply. Thus, the field tests described above, especially that developed for WFP's Blue Box, would be applicable. If aptamer-based kits could be developed with long shelf-lives, they could be part of an early-warning and

rapid-response system that could be available for deployment on short notice to a suspected outbreak of mycotoxicosis. The workshop sponsored by the CDC and WHO in response to the aflatoxicosis outbreaks in Kenya provided an outline of a surveillance and monitoring system involving weather, food, and animal and human health parameters that could be used as part of such an early warning system (20). Localized testing with suitable field kits would add to the value of such a system, especially if a kit for the measurement of a biomarker were to be developed.

Conclusions

African communities remain at risk for mycotoxin toxicoses, particularly aflatoxicosis. Increased awareness in health professionals may result in wider recognition of and more rapid notification for these food-borne disease outbreaks, although it is hoped that the current projects by PACA will improve food safety with respect to aflatoxin. In meeting the challenge, appropriate fit-for-purpose analytical responses should be available. Current technology is adequate to meet the challenge, although it may require adaptation and development for in-field applications.

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