Data on the prevalence of mycotoxins in staple foods are essential for all applied research into their impact on health and on effective mitigation. Country- or region-specific knowledge enables the identification of susceptible edible crops that are responsible for toxin exposure in specific populations. Prevalence data can indicate how effective maximum levels have been in influencing food safety, while acknowledging that their enforcement could have food security implications. Monitoring of prevalence also provides information on how various implemented strategies to reduce contamination or exposure levels directly affect toxin levels.

Ideally, exposure assessment, as one component of risk assessment, integrates mycotoxin levels with food consumption patterns and thus provides, via risk characterization, a clear picture of the extent to which mycotoxins compromise food safety and health, at either an individual or a population level. However, this is generally not achieved in developing countries, primarily due to a lack of country-specific data, resources, and analytical capacity.

Exposure biomarkers, such as serum aflatoxin–albumin adducts (AF–alb) or urinary fumonisin B₁ (UFB₁), offer a more integrated estimate of exposure from all sources for either aflatoxin or fumonisin, and offer potentially more reliable exposure estimates. Measurement of exposure, either by measures of food consumption combined with contamination levels or by using biomarkers of exposure, can be used to identify the main dietary contributors to exposure, detect areas with unacceptable exposures, assess health impacts of mycotoxins and their role in disease development, and determine the efficacy of intervention strategies. The recent development of multitoxin analytical methods, whether applied to food or to biological samples as biomarkers, has raised awareness of the concurrent exposure to aflatoxin and fumonisin as well as sometimes to other, unanticipated mycotoxins.

**Exposure to aflatoxins**

Aflatoxins are mycotoxins found in four main forms: aflatoxin B₁ (AFB₁), B₂ (AFB₂), G₁ (AFG₁), and G₂ (AFG₂). Aflatoxins occur on a wide range of crops, including the major staple cereals (e.g. maize), edible nuts and legumes, and their products. In general, AFB₁ occurs at the highest levels and is the most toxic. The main fungal producers of aflatoxins are *Aspergillus flavus*,
which produces AFB<sub>1</sub> and AFB<sub>2</sub>, and *Aspergillus parasiticus*, which produces all four forms. Contamination can occur before or after harvest or both.

Aflatoxin contamination levels can vary widely, from products that meet the strict maximum levels set by the European Commission (2 µg/kg for AFB<sub>1</sub>; 4 µg/kg for total aflatoxins [sum of AFB<sub>1</sub>, AFB<sub>2</sub>, AFG<sub>1</sub>, and AFG<sub>2</sub>] for cereals and nuts for direct human consumption) (European Commission, 2010) to products with levels that can pose a risk of acute aflatoxicosis. For example, determination of total aflatoxins in a rural market survey in four districts during an acute outbreak in Kenya, in 2004, showed a range of total aflatoxins of 1–46 400 µg/kg, with 7% of samples above 1000 µg/kg (Lewis et al., 2005). In 2003, data available from African countries were summarized by Shephard (2003). More recent data, including summaries of global occurrence in samples submitted for analysis, have been presented by Rodrigues et al. (2011) and Schatzmayr and Streit (2013). Recent African data have also been provided by Gnonlonfin et al. (2013). Examples from this literature include groundnut cake from Nigeria (range, 20–455 µg/kg); raw groundnut from Kenya (non-detectable to 20–455 µg/kg); and maize from Benin (2–2500 µg/kg), Ghana (20–355 µg/kg), and Zambia (1–109 µg/kg). Other aflatoxin-contaminated food sources reported in various African countries include cassava, tiger nuts, cowpeas, sorghum, okra, and hot peppers, although due to consumption patterns, maize and groundnuts dominate in terms of level of exposure.

Aflatoxin M<sub>1</sub> (AFM<sub>1</sub>) is a toxic metabolite of AFB<sub>1</sub> and a possible human carcinogen (IARC, 2012). This compound can be detected in the urine and milk of exposed animals, including humans. Data on the carryover of AFM<sub>1</sub> to breast milk are limited, but the carryover has been estimated at 0.1–0.4% (Zarba et al., 1992), and exposure of infants to AFM<sub>1</sub> from human breast milk has been reported in developing countries (Shephard, 2004; Turner, 2013; Magoha et al., 2014). In addition, AFM<sub>1</sub>, from milk of livestock consuming AFB<sub>1</sub>-contaminated feed is a further source of exposure. The 56th meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) compiled data on AFM<sub>1</sub> levels found in commercial raw and processed dairy milk (Henry et al., 2001). However, few data were available from Africa, and those reported are unlikely to reflect typical village- or subsistence farm-level exposures. Further study is needed to better understand the consequences of AFM<sub>1</sub> ingestion from breast milk and/or from the milk of livestock in Africa.

Global intake estimates for aflatoxin (ng/kg body weight [bw]/day) have been reported based on estimates of typical maize and nut consumption, contamination levels, and body weight (Liu and Wu, 2010). For Africa, estimates were made for the Democratic Republic of the Congo (range, 0–27), Ethiopia (1–36), The Gambia (4–115), Kenya (4–133), Mozambique (39–180), Nigeria (139–227), South Africa (0–17), the United Republic of Tanzania (0–50), and Zimbabwe (18–43). Similarly high intakes were reported for China and countries in South-East Asia, compared with western Europe and North America at 0–1 ng/kg bw/day (Turner et al., 2012; Schleicher et al., 2013). These data indicate a much higher burden of exposure in low-income regions. However, it is important to note that these estimates are based on very limited data-sets, particularly in those regions at greatest risk of high exposures.

**Exposure to fumonisins**

Fumonisins, which are produced mainly by *Fusarium verticillioides* (Sacc.) Nirenberg and *F. proliferatum* (Matsush.) Nirenberg, are common contaminants of maize and maize-based products. Fumonisin B<sub>1</sub> (FB<sub>1</sub>) is the most abundant (generally ~70% of the total fumonisin contamination), and it normally co-occurs with lesser amounts of fumonisin B<sub>2</sub> (FB<sub>2</sub>) and B<sub>3</sub> (FB<sub>3</sub>). Occurrence on sorghum has also been reported (Bulder et al., 2012).

Fumonisins were evaluated by JECFA in 2001 and 2012 (Bolger et al., 2001; Bulder et al., 2012). As exposure is a product of both contamination level and consumption, certain rural communities in developing countries can exceed the provisional maximum tolerable daily intake (PMTDI) of 2 µg/kg bw/day of fumonisin if their diet contains high amounts of maize (Burger et al., 2010).

Fumonisin intake estimates (µg/kg bw/day) in several regions of Africa were recently reviewed (Wild and Gong, 2010), including Burkina Faso (0–2); Bizana (1–19); Cen-tane (2–36), Transkei (4), and Kwa-Zulu-Natal (0), South Africa; and Bomet, Kenya (<0.1). Intakes of 0.2–26 µg/kg bw/day in Tanzanian children were reported (Kimanya et al., 2014).

In Latin America, estimates of fumonisin intake in Guatemala were reported to be 3.5 µg/kg bw/day (urban) and 15.5 µg/kg bw/day (rural) (Wild and Gong, 2010), and more recently a range of 0.20–23 µg/kg bw/day was reported (Torres et al., 2014).
Biomarkers for aflatoxins and fumonisins

Food contamination and food intake can vary greatly within rural subsistence farm settings and between villages and individuals. Assessments of both of these parameters present analytical and measurement difficulties. In addition, there is individual variation in toxicokinetics and toxicodynamics related to toxin ingestion. For these reasons, considerable effort has been given to developing biomarkers for aflatoxins and fumonisins (Turner et al., 2012).

For AFB₁, the peripheral blood AFB₁ biomarker has been validated for moderate- to long-term exposure (several months), whereas the urinary biomarkers, aflatoxin–N7-guanine and AFM₁, reflect shorter exposures. The application of these biomarkers has helped establish the link between aflatoxin exposure and the development of liver cancer (Kensler et al., 2011; IARC, 2012) and has allowed the efficacy of intervention studies to be demonstrated (Turner et al., 2005).

Validated aflatoxin biomarker data from sub-Saharan Africa show that the ranges of exposures are likely to vary greatly in many regions and within and across closely located villages and agro-ecological zones, as well as seasonally and annually (Turner et al., 2012; Turner, 2013). The biomarker data further highlight the early-life burden of exposure, including in utero and during early infancy. Exposures in West African studies involve both maize and groundnuts as the primary sources of intake of aflatoxins. Typical biomarker levels in children younger than 5 years in Benin, The Gambia, and Togo range up to 1000 pg aflatoxin–lysine/mg albumin (Turner, 2013). By comparison, levels of AFB₁–alb reported from the recent United States National Health and Nutrition Examination Survey (NHANES) were almost all (99%) below the limit of detection (LOD), and the geometric mean of the positives was only 0.8 pg/mg (Schleicher et al., 2013).

AF–alb has also been used in various studies to assess associations between aflatoxin exposure and infant and early childhood growth faltering (Turner, 2013). Typically there is greater confidence in the long-term markers of aflatoxin exposure to assess health outcomes, as they provide an integrated measure over several months. Several putative biomarkers for fumonisin exposure have been investigated. These include sphingoid bases in plasma and urine and FB₁ in hair, nails, serum, urine, and faeces (Shephard et al., 2007); however, none of these have been validated in human studies. UFB₁ has been measured in human samples in regions with known high exposure to dietary fumonisins (Gong et al., 2008a; Xu et al., 2010; van der Westhuizen et al., 2011; Riley et al., 2012; Torres et al., 2014). In general, statistically significant relationships between UFB₁ and either estimated or measured FB₁ intakes were reported; however, the data indicate that the urinary measurement was only moderately reflective of the level of intake.

Co-occurrence of aflatoxins and fumonisins

The co-occurrence of aflatoxins and fumonisins has been widely documented by both biomarker studies and food analyses. In the United Republic of Tanzania, AF–alb and UFB₁ were assessed in young children (Shirima et al., 2013). The prevalence of detection of both of the mycotoxins was high, and 82% of the children were positive for both. Also, a modest but statistically significant correlation was observed between the concentrations of these biomarkers ($r = 0.375, P < 0.001$) (Shirima et al., 2013). Urinary aflatoxin and fumonisin were observed less frequently in samples from two major cities, Yaoundé and Bamenda, in Cameroon (Abia et al., 2013) and from rural regions of Nigeria (Ezekiel et al., 2014), although co-exposures did occur. Differences in the sensitivities of the analytical methods between these studies limit direct comparison. A separate study from Cameroon, looking at urinary mycotoxin markers in young children, also reported aflatoxin and fumonisin exposure (Njumbe Ediage et al., 2013). These data were complemented by a survey across multiple agro-ecological zones in Cameroon, in which maize, groundnuts, and cassava were found to be contaminated with multiple mycotoxins (fumonisins were found in 74% of the maize samples and aflatoxins in 22% of the maize, 29% of the groundnuts, and 25% of the cassava samples) (Ediage et al., 2014). In a study by Probst et al. (2014), a total of 339 maize samples from 18 countries in Africa were assessed for aflatoxin and fumonisin contamination. Aflatoxins were detected (LOD, 1 µg/kg) in 47% of the samples, with 7% exceeding 20 µg/kg and 6% exceeding 100 µg/kg (the maximum level was 1409 µg/kg). Fumonisins were detected (LOD, 500 µg/kg) in 81% of the samples, with 7% exceeding 5000 µg/kg and 3% exceeding 100 000 µg/kg. Aflatoxin and fumonisin co-contamination occurred in 35% of the samples. Concentrations of co-contaminants varied by region, but for the Coast Province in Kenya, for example, 50% of samples contained high levels of both aflatoxins (mean, 97 µg/kg) and fumonisins (mean, 32 000 µg/kg) (Probst et al., 2014).
In Latin America, co-exposures to aflatoxins and fumonisins have also been documented. Maize from 22 districts in Guatemala was analysed; 36% of 572 samples tested positive for aflatoxins (mean, 63 µg/kg; range of positives, 5–2655 µg/kg), and 99% of 640 samples tested positive for fumonisins (mean, 1800 µg/kg; range of positives, 10–17 000 µg/kg) (Torres et al., 2015).

Analytical limitations

One limitation with urinary biomarker approaches is the volumes of urine required. Even though technological development of highly sensitive liquid chromatography-mass spectrometry (LC-MS) techniques will help support biomonitoring, the approach itself may be limited by instrumentation costs, restricting analysis to specialist laboratories. With the development of multitoxin analytical techniques based on LC-MS/MS, multibiomarker methods have been developed for urinary biomarkers for toxins, including FB1 and AFM1 (Solfrizzo et al., 2011; Warth et al., 2012), as extensions of multimycotoxin methods for food analysis. These methods have been applied in Africa to evaluate exposure (Abia et al., 2013; Shephard et al., 2013; Ezekiel et al., 2014). To date, there have been limited efforts to compare multimycotoxin methods from different laboratories. Thus, currently there is greater confidence in the data from single measures, and for increased utility these inter-laboratory comparison studies are urgently needed. An additional concern is that some of the multimycotoxin methods, especially for foods, may be measuring contaminants of limited relevance to human health. This could result in additional costs (e.g. of measuring > 60 metabolites) while potentially leading to inaccurate measurements.

Key scientific gaps

The problem of mycotoxin exposure is most acute in developing countries, which lack resources and analytical capacity for analyses. Consequently, few data are reported from developing countries and those available are usually based on only a limited number of samples of uncertain quality. As a result, there is a widening gap between the quality and quantity of prevalence data generated by laboratories in developed countries compared with developing countries. There is thus a need in the developing countries to have sampling and analytical tools available that are fit for specific purposes, such as:

- A rapid screening method aimed at the field/subsistence farm level that is inexpensive and user-friendly and has a wide dynamic analytical range. This could additionally help support a rapid alert system that informs responses and appropriate actions for food safety.
- A comprehensive regional or country-wide monitoring programme, involving the establishment of a reference laboratory within a country/region. The monitoring programme should be developed within existing surveillance systems and be expanded over time. For example, many regions have national health and nutrition programmes where archived biospecimens could be requested. Future national surveys of this nature may be asked to collect larger volumes of biospecimens (e.g. to support urinary xenobiotic surveillance). De novo monitoring activities could include both food measures and biomarkers.

For a successful food monitoring programme, it is essential to have effective sampling plans in place. While it is recognized that designing effective sampling plans for mycotoxin detection in food commodities is a complex task, there is a tool available to support countries in this regard: the Food and Agriculture Organization of the United Nations (FAO) Mycotoxin Sampling Tool (http://www.fstools.org/mycotoxins/). Further, there is a World Health Organization (WHO) programme (Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme [GEMS/Food]) that collects global food contamination data and reports food consumption data. Average per capita food consumption data are reported based on the FAO Food Balance Sheet data. It is important to note that the database provides average consumption levels but will not capture the food consumption pattern at the subsistence farm level. Another database within GEMS/Food collects occurrence data for contamination levels, including aflatoxins and fumonisins in food products and crops. It would be useful to highlight the opportunity for researchers to add their studies to this database. However, acquiring data on consumption and contamination levels in subsistence farmers will remain a significant hurdle.

Among monitoring options, an approach that might be implemented is sampling at community maize milling facilities. For example, in some parts of East Africa farmers could bring maize to a local milling operation, where subsampling and aflatoxin and fumonisin analyses could be carried out using rapid test kits for field application. Relatively large data collection activities may be possible in such settings, providing an improved surveillance, although this will capture only some of the prevalence data in some regions and none in others. This also may, however, provide a target site for intervention.
Measures of individual exposures are important for epidemiological investigations of disease causation and for demonstration of efficacy of intervention. The development of a reliable source of certified standards, especially for aflatoxin biomarkers, would allow a substantial increase in biomarker-directed epidemiology research.

Therefore, the problem of insufficient data could also be addressed by the use of individual biomarkers of exposure. Aflatoxin biomarkers are well understood, but the most useful for long-term exposure studies, AF–alb, is currently measured in only a limited number of laboratories. It would be advantageous if this analysis were more generally available, especially in countries where aflatoxin exposure is known to be high. The lack of reagents such as aflatoxin–lysine and mono-adducted AF–alb is a major constraint and needs to be addressed. Enzyme-linked immunosorbent assay (ELISA) approaches are typically less expensive, but an additional issue is a lack of commercially available kits or antibodies. While LC-MS provides robust data, the analytical costs are prohibitive for most laboratories. Exposure of infants in developing countries to AFM1 also needs to be monitored as these countries are prone to higher AFB1 exposures.

UFB1 has been measured by LC-MS in several world regions, and again a current concern is the cost of the analysis. While dose–response relationships were reported, the urinary measure was not as strongly predictive of the level of intake compared with relationships reported for aflatoxin biomarkers. For general biomonitoring this is not a major issue; however, this is a concern when making assessments in relation to putative health effects and assessing the efficacy of interventions. For the use of FB1 and AFM1, it was noted that neither of these predicts longer-term exposures, and while serum AF–alb is used for this purpose in aflatoxin biomonitoring and epidemiology, there remains a need to develop a long-term exposure biomarker for fumonisins. An additional challenge is the need for higher-throughput analytical tools, which would benefit from a cooperative activity between experts in exposure assessment and researchers with subject matter expertise in mycotoxins.