Chapter

Aflatoxin B1: Chemistry, Environmental and Diet Sources and Potential Exposure in Human in Kenya

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Abstract

Cancer incidences and mortality in Kenya are increasing according to recent reports and now number among the top five causes of mortality in the country. The risk factors responsible for this increase in cancer incidences are assumed to be genetic and/or environmental in nature. The environmental factors include exposure to carcinogenic contaminants such aflatoxins (AFs). However, the exact causes of the increase in cancer incidences and prevalence in many developing countries are not fully known. Aflatoxins are known contaminants produced by the common fungi *Aspergillus flavus* and the closely related *Aspergillus parasiticus* which grow as moulds in human foods. Aflatoxin B1 (AFB1) is most common in food and is 1000 times more potent when compared with benzo(a)pyrene, the most potent carcinogenic polycyclic aromatic hydrocarbon (PAH). Aflatoxins have therefore drawn a lot of interest in research from food safety and human health point of view. In this chapter, the chemistry, synthesis, identification, toxicology and potential human health risks of AFB1 in Kenya are discussed.

Keywords: aflatoxin B1, chemistry, determination, toxicity, exposure, health risks, Kenya

1. AFB1 chemistry

The aflatoxins were discovered in a toxic peanut meal after causing 'turkey X' disease, which killed large numbers of turkey poults, ducks, young pheasants and chicks in the UK in the early 1960s [1], and more than 100,000 young turkeys in poultry farms were killed [2]. The peanut meal was highly toxic, and the toxin-producing fungi was identified as *Aspergillus flavus* hence the name of the toxin, aflatoxin [2]. Extracts of the feed later induced the now known toxic symptoms in experimental animals, and purified metabolites with properties identical to aflatoxins B1 and G1 (AFB1 and AFG1) were later isolated from the *Aspergillus flavus* cultures [1, 3, 4].

Structural elucidation of aflatoxins was accomplished and confirmed by total synthesis in 1963 [4]. There are four major aflatoxins B1, B2, G1 and G2 plus two additional toxic metabolic products M1 and M2 that are of significance as direct contaminants of foods and feeds and whose structures have been elucidated [3, 4].

These toxins have similar structures and form a unique group of highly oxygenated, naturally occurring heterocyclic compounds [5]. Their structures and molecular formulae are shown in **Figure 1**. *Aspergillus flavus* typically produces aflatoxin B1, which is the most potent and the most frequently identified in aflatoxin contaminations, and aflatoxin B2, whereas *Aspergillus parasiticus* produces aflatoxin G1 and aflatoxin G2 as well as aflatoxin B1 and aflatoxin B2. Four other aflatoxins M1, M2, B2A and G2A [3, 6], which are produced in minor amounts, were subsequently isolated from cultures of *Aspergillus flavus* and *Aspergillus parasiticus*. Aflatoxins M1 and M2, which are found in milk of animals that have consumed feeds contaminated with AFB1, are the hydroxylated metabolites of aflatoxins B1 and B2, respectively [3, 7]. Aflatoxins are, in essence, known as a group of mycotoxins which are produced primarily by some strains of *Aspergillus flavus* and by most strains of *Aspergillus parasiticus*, plus related species of *Aspergillus niger*, among others [8].

Aflatoxins are just a subset of class of mycotoxins which are fungal metabolites rampant and invisible in the environment and have caused severe effects on food security and safety especially within sub-Saharan African (SSA) societies [9]. This class of mycotoxins include *Fusarium* mycotoxins which have been found in oesophageal cancer-prone areas of South Africa [10], aflatoxins, fumonisins and ochratoxin A which have all been found to be rampant across West, East and Central Africa [11, 12]. Aflatoxins have become the most common and ubiquitous food contaminants produced by the common fungi *Aspergillus flavus* and the closely related *Aspergillus parasiticus*.

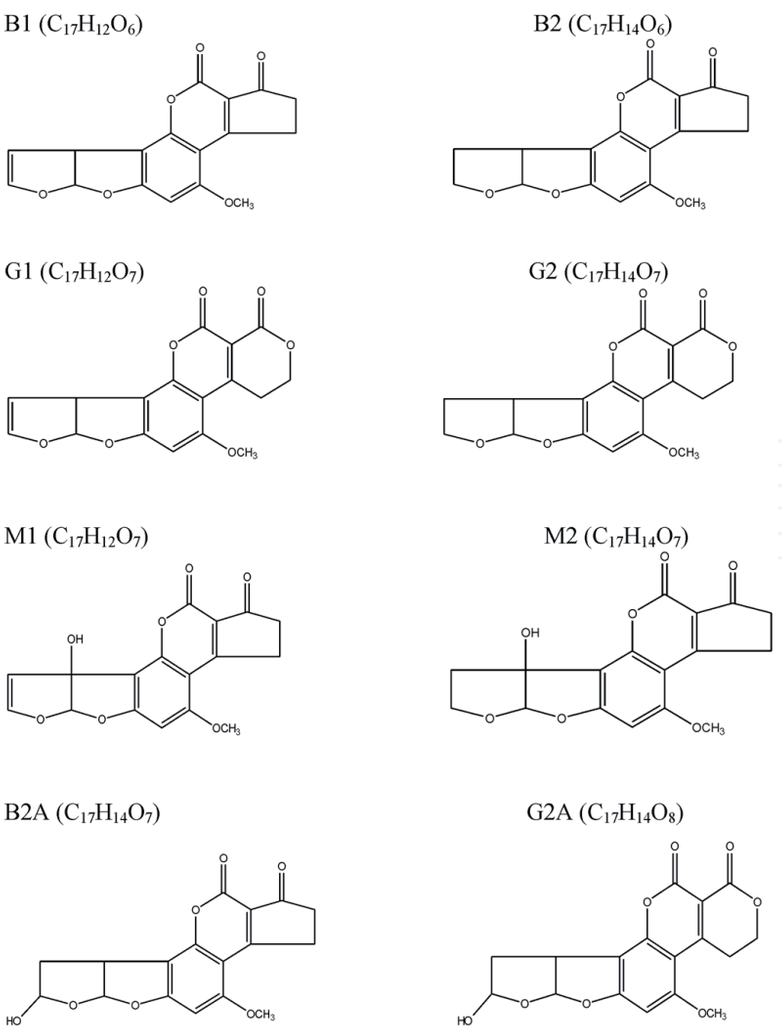

Figure 1.
Chemical structures of aflatoxin B1 and other related aflatoxin metabolites [3, 6].

Aflatoxin B1 (AFB1) is a secondary metabolite produced by *Aspergillus flavus* and *Aspergillus parasiticus* when environmental factors are favourable [13, 14]. It has also been characterized as a biological toxin. Biological toxins are defined as toxic substances produced by microorganisms, animals and plants that have the capability of causing harmful effects when inhaled, ingested, injected or absorbed (medical dictionary). Referring to **Figure 1**, all aflatoxins are heterocyclic compounds which have a common benzene ring, with slight variations only in terms of the presence of double bonds and ketonic groups and the metabolites having hydroxy groups, with hydroxylation positions varying from one metabolite to another. These structures indicate slight aqueous solubility and ease of epoxidation reaction, respectively, which are considered to influence both their excretion and toxicity. AFB1 which is the most prevalent and most potent, a human health hazard globally, has a peculiar double bond in the cyclic ring which is also observed in G1 and M1. For activation, AFB1 requires epoxidation to aflatoxin B1 2,3-epoxide. The microsomal cytochrome P450 (CYP450) monoxygenases biotransform the toxin to the less toxic metabolites aflatoxins M1 and G1 [5]. Aflatoxins are highly oxygenated and naturally occurring heterocyclic compounds [4] which have been separated based on their fluorescence under UV light and the presence or lack of a double bond at the 8, 9 carbons. Aflatoxins B1 and G1 have a double bond at the 8, 9 carbons, which allows for formation of an epoxide, a more toxic form of AFB1 and AFG1, while AFB2 and AFG2 do not. Aflatoxins B2 and G2 were established as the dihydroxy derivatives of B1 and G1, respectively. Whereas, aflatoxin M1 (AFM1) is 4-hydroxy aflatoxin B1, aflatoxin M2 is 4-dihydroxy aflatoxin B2 [5]. Hydrogenation of B1 and G1 yields B2 and G2, respectively.

The important physico-chemical properties of AFB1 are shown in **Table 1**. It is odourless, tasteless and colourless. It is difficult to detect sensorically, and therefore it poses a real challenge to food handlers, consumers and regulators who are in a bid to control or eradicate it [15–17]. AFB1 exists as colourless to pale yellow crystals or white powder [18]. Aflatoxins are densely fluorescent; B refers to blue fluorescence, while

Physico-chemical property	
IUPAC name	2,3,6a,9a-Tetrahydro-4-methoxycyclopenta[c] Furo[3′,2′:4,5]furo[2,3-*h*][l] benzopyran-1,11-dione
MW	312.277 g/mol
mp	268–269°C
Physical state	Colourless pale yellow crystalline to solid or white powder; odorless
Specific Optical rotation	−558 °/D at 25°C (0.1 M in chloroform) or −480 °/D at 25°C (0.1 M in dimethyl formamide)
Vapour pressure	2.65×10^{-10} mmHg at 25°C
Water solubility	16.14 mg/l at 25°C; decreases at low temperature; generally soluble in water and polar solvents
Stability	Stable until melting point; decomposed by UV irradiation in water/chloroform
Log K_{ow}	1.23
BCF (fish)	3
Koc (soil)	Ranges within 682–2.317×10^{-4}
Henry's law constant	1.4×10^{-13} atm m^3/mol at 25°C
Fluorescence emission	Densely fluorescent blue (λmax = 450 nm)
UV absorption	Absorbs at 223, 265 and 362 nm
Mass spectrum	Identified by LC–MS; ionization ESI; precursor-type $[M + H]^+$; m/z 313.071

Table 1.
Physico-chemical properties of AFB1.

G signifies green fluorescence. AFB1 exhibits a blue fluorescence with a fluorescence emission spectrum maximum of 425 nm and has UV maximum absorbance values at 223, 265 and 362 nm (in ethanol). It strongly absorbs UV light and is decomposed by it when dissolved in water or chloroform or when it is in form of solid films. AFBI has a Henry's law constant value of 1.40×10^{-13} atm m^3/mol at 25°C and a vapour pressure of 2.65×10^{-10} mmHg at 25°C. These properties would enable it to be less volatile and therefore has become very ubiquitous in the environment, becoming distributed in air, water and soil [15, 18]. It therefore can spread easily on the farm or in stores causing heavy damage to agricultural food crops and stored grains, respectively.

The vapour pressure of AFB1 indicates that AFB1 will tend to exist solely in particulate phase in the atmosphere if released into air, according to a model of gas/particle partitioning of semivolatile organic compounds [19]. The particulate bound AFB1 will then tend to be removed from the atmosphere by wet and dry deposition. Since it absorbs UV light, it is susceptible to direct photolysis by sunlight. If released to soil, AFB1 is expected to have low mobility based on its K_{oc} value which ranges from 682 to 2.3×10^4 and Freundlich adsorption coefficients, ranging from 17 to 238 mg/kg in different soil types. Volatilization from moist soils or water surfaces is not expected to be an important fate process based on its Henry's law constant value of 1.4×10^{-13} atm-cm/mol. It is also not expected to volatilize much from dry soil surfaces based on its vapour pressure which is very low. The K_{oc} of AFB1 indicates that it is expected to adsorb to soil and sediment. However, based on its K_{ow} and BCF values, AFB1 would tend to have a relatively moderate potential for bioconcentration in aquatic organisms and animal adipose tissue. Perhaps this explains why it is rapidly absorbed in the stomach and intestines and why it is present in the blood, kidney and liver where it imparts its toxicity. In the water environment, AFB1 can undergo hydrolysis as it contains a cyclic ester functional group and the rates of hydrolysis are similar to those of non-cyclic esters, ranging from months to a year under normal environmental conditions (i.e. pH 5–9) [19]. However, ring strain and steric hindrance have been reported to prevent its ease of hydrolysis, and therefore the extent of hydrolysis is unexpectedly low [18]. AFB1 biodegradation in soil and water has been studied, and it has been found that biodegradation may not be a very important environmental fate process. For example, after incubation for 120 days in silt loam, clay loam and sandy loam soil types, respectively, only 8.1, 4.9 and 1.4% complete mineralization to CO_2 was achieved [19]. Biodegradation in various soils with different pHs (ranging 5.8–7.3), organic carbon (OC) (ranging 0.46–2.82%) and cation exchange capacity (CEC) (ranging 11.7–18) showed very low concentrations of metabolites B2 and G2 after 1 day in a 20-day experiment, and the TLC results indicated that adsorption onto soil prevented AFB1 decomposition.

Biotransformation of aflatoxins has been studied and found to occur via four main routes [19–23]: (i) hydroxylation of carbon atom at junction of the two fused furan rings, aflatoxin B1 is converted into AFM1, and this occurs to some extent in the mammalian liver [19, 20]; (ii) oxidative o-demethylation of single aromatic methoxy-substituent gives aflatoxin P1 [19]; (iii) hydration of vinyl double bond would afford hemiacetals, and aflatoxin B1 has been converted to into hemiacetal AFB2A in pig, mouse and avian livers through this route [19, 22] and (iv) reduction of cyclopentenone ring, dihydroaflatoxicol, but this biotransformation seems to be confined to avian species and not mammals [19]. While the hydroxylated metabolite AFM1 is the product of metabolism of AFB1 and AFB2, G1 and G2 were established as dihydroxylated derivatives of B1 and B2, respectively. AFM1 is 4-hydroxy aflatoxin B1 and AFM2 is 4-hydroxy aflatoxin B2. The order of acute and chronic toxicity is B1 > FG1 > B2 > G2 [20].

Extensive studies on reactions of aflatoxins to various physico-chemical conditions and reagents have been conducted because of possible application of such

reactions in detoxification of materials contaminated with aflatoxins [24]. In dry state, aflatoxins are heat stable up to melting point. However, in the presence of moisture and elevated temperatures, aflatoxins are destroyed to certain extents over a period of time. Such destructions of aflatoxins have been found to occur in oil seeds, meals and roasted peanuts or in aqueous solution at pH 7 [15–17]. It is postulated that such treatments can lead to the opening of the lactose ring, with possible destruction of decarboxylation, at elevated temperature [21]. In alkaline solution, hydrolysis of the lactose ring occurs, but this hydrolysis appears reversible, since it has also been shown that recyclization occurs following acidification of basic solutions containing aflatoxin [21, 24]. At a temperature of 100°C, lactose ring opening can occur, followed by a decarboxylation reaction [21]; and this reaction can further lead to a loss of the methoxy group from the aromatic ring [22]. In the presence of mineral acids, aflatoxins B1 and G1 are converted to aflatoxins B2A and G2A, respectively, due to acid-catalyzed insertion of water molecules across the double bonds in the furan ring, leading to hydroxylation (see **Figure 1** chemical structures). In the presence of acetic and hydrochloric acids, the reaction of AFB1 and AFG1, respectively, gives the acetoxyl derivatives, with acetoxyl groups attached on the benzene rings [22]. Similar adducts of aflatoxins B1 and G1 are formed with methanoic acid-thionyl chloride, acetic acid-thionyl chloride and trifluoroacetic acid [22]. Reactions with oxidizing agents, such as sodium hypochlorite, potassium permanganate, chlorine, hydrogen peroxide, ozone and sodium perborate, change the aflatoxin molecule in some way as indicated by loss of fluorescences although the mechanisms of these reactions are still uncertain as the products remain unidentified in most cases [25]. Hydrogenation of aflatoxins B1 and G1 yields aflatoxins B2 and G2, respectively. If further reduced by 3 mol of hydrogen, aflatoxin B1 yields tetrahydroxyl aflatoxin, while reduction of aflatoxins B1 and B2 with sodium borohydride yields aflatoxins RB1 and RB2, respectively. The RB1 and RB2 arise because of the opening of the lactose ring followed by reduction of the acid group and the keto group in the cyclopentane ring. However, it should be noted that breakdown of aflatoxins by various means does not guarantee safety of the contaminated substance. At times this breakdown is reversible or may lead to another form of aflatoxin. Besides, reaction products have not been subjected to detailed examination, including length of time the reactions take place [25]. Researchers have just concluded that the decomposition is not complete based on trials with food samples [26].

In general, the aflatoxins have been considered as difuranocoumarins, which are highly substituted coumarin derivatives containing a fused dihydrofurofuran moiety [1, 3, 4]. In particular, AFB1 is characterized by the fusion of a cyclopentenone ring to the lactone ring of the coumarin structure (**Figure 1**) and by strong fluorescence emission in the blue region (hence the designation B) when exposed to ultraviolet light [1, 3, 4]. Aflatoxins Bs strongly emit blue colour when they absorb UV light, and aflatoxins Gs strongly emit green colour when they absorb UV light. AFM1 is the principal hydroxylated metabolite of AFB1 and is produced upon the action of cytochrome P450 1A2 (CYP1A2) [27, 28]. It is strongly fluorescent, emitting blue-violet light. Specifically, AFB1 has similar chemical properties to other metabolites which include its slight solubility in water and polar organic solvents and less solubility in nonpolar solvents [23]. It has strong thermal stability, even at high temperature (>100°C), and this prevents it from being thermally degraded completely during food manufacturing, for example, when milk and dairy products are processed, since pasteurization and other thermal treatment methods alone are ineffective [29, 30]. Other chemical properties of AFB1, such as its instability to UV light or extreme pH conditions (<3 or >10) and reactivity of lactone moiety in the presence of ammonia or hypochlorite, have been useful in the development of methods for decontamination of feed and food [29, 30]. Several physical treatment

methods like exposure to microwaves, gamma rays, X-rays and ultraviolet light have been investigated, but inconsistency of the results has discouraged their use, especially for heavily contaminated samples [31]. At present, ammoniation [32] and adsorption on clays or organic adsorbents [29] have commonly been used to achieve a good level of decontamination without disruption of the nutritional properties or safety of feed.

Biological methods of detoxification of mycotoxins are of two different types: the first being via enzymatic degradation and the second via sorption. In enzymatic biochemical processes, live microorganisms can biodegrade and mineralize the mycotoxins completely to CO_2 or absorb them by attaching them to their cells by active interaction and accumulation and thereby reducing them from the media. Dead organisms can adsorb mycotoxins, and they can be used to make biofilters for fluid decontamination of products, where the aflatoxins are left on the filter and the products become subsequently decontaminated, or as probiotics to bind and remove mycotoxins from the human intestine [15, 33]. Enzymatic degradation can be complete mineralization to CO_2, in which either extracellular or intracellular enzymes and various species of bacteria have been identified including *Pseudomonas*, *Bacillus* and *Lactobacillus* and used to inhibit toxicity or production of aflatoxins by *Aspergillus*. A large number of microorganisms (approximately 1000) have been screened for this purpose, but only *Lactobacillus* have been adopted [34, 35]. AFB1 and AFM1 have been shown to have a strong binding ability to other molecules, and recently research has been focusing on the AFB1-binding capacity to certain metabolites, for example, different strains of *Lactobacillus* in milk for aflatoxin decontamination in different products such as yoghurt [34, 36, 37].

Various chemical treatment processes have been tried, including sodium hypochlorite (NaOCl), potassium permanganate ($KMnO_4$), hydrogen peroxide (H_2O_2), sodium bicarbonate, sodium chloride and sodium borohydride ($NaHBO_3$) a well-known reducing agent, to detoxify or decompose aflatoxins in various foods [16, 38, 39]. These reagents can be used, and, for example, formaldehyde and NH_3 were found to neutralize AFB1, while $NaSO_4$ was found to be less efficient in neutralizing AFB1 [38]. However, these reactions have to be optimized in terms of quantities needed and reaction time as well as temperature and pressure conditions required. Different cooking methods have also been tried to remove aflatoxins from foods [16, 17, 38, 40]. Normal cooking of rice was found to destroy only 49% AFB1 [16, 17]. In other experiments to study the reduction of aflatoxins in various products, boiling of maize in traditional cooking used in Kenya destroys 11–17.6% AFB1 and AFG2 [40], while in beer making 18–27% AFB1 still remain [38] and in bread making 25% still remain [26]. Kirui [39], in assessing the levels of aflatoxins that were left after various treatments following physico-chemical and traditional cooking methods for maize and maize products, found that boiling maize reduced total aflatoxin level from 83 to 7 ppb, dry decortication reduced the level from 51.3 to 9.6 ppb, boiling with Magadi soda (food softener) reduced the level from 59 to 13.4 ppb, solar irradiation (18 h) reduced the level from 60.8 to 13.7 ppb and UV irradiation (18 h) reduced the level from 81.7 to 61.4 ppb. He found that only dry decortication method, which involves boiling with Magadi soda followed by washing with water and boiling, respectively, reduced the levels significantly but not completely below the maximum limits. Alkali treatment with inorganic (e.g. boiling with NaCl) and organic bases were reported to be effective and economically feasible [17]. Occupational exposure to AFB1 has been reported to occur through inhalation and dermal contact at work places where commodities such as peanuts, grains, linseed oil or animal feeds are produced, stored or used. An average AFB1 exposure of 64 ng/d-kg body weight was reported for Danish workers in the animal feed production industry. General population may most likely be exposed to AFB1 via ingestion of contaminated food [18].

2. Synthesis of aflatoxin B1

The biosynthetic pathway of AFB1 has been explained by researchers. It is derived from both a dedicated fatty acid synthase (FAS) and a polyketide synthase (PKS) which occur in the mould, together known as norsolorinic acid synthases. The biosynthetic pathway has been described by Singh and Hsich [41], Yu et al. [42] and Dewick [43], among others, and, an outline of the method can be found in Wikipedia. The process begins with a FAS-aided synthesis of hexanoic acid, which is the starter unit for the iterative type I PKS. A PKS catalyzes addition of seven malonyl-CoA molecules to the hexanoic acid to form a C20 polyketide compound. The polyketide folds through a cyclization process induced by a PKS to form an anthraquinone norsolorinic acid, and a reductase enzyme then catalyzes the reduction of the ketone on the norsolorinic acid side chain to yield an intermediate, an averantin [41–43]. From here, various processes which are assisted with different enzymes including hydroxylases, dehydrogenases (for oxygenation and cyclization), CYP450 oxidases, esterases, reductases, methyl transferases and oxidoreductases occur, leading to different intermediates. The pathway for AFB1 biosynthesis is very complicated, and some of the enzymes and intermediates involved continue to be elucidated and characterized [43].

Under favorable moulding conditions, *Aspergillus flavus* spores germinate by attaching their mycelium in a food substrate and secreting enzymes which break down nutrients into simpler compounds capable of digestion. During digestion, *Aspergillus flavus* then produces, as described in the foregoing paragraph, secondary metabolites, including AFB1, meant to give the fungi a competitive edge against other microorganisms [44].

For research and other purposes, aflatoxins can be produced in small quantities by fermentation of *Aspergillus flavus* or *Aspergillus parasiticus* on solid substrates or media [45]. It is extracted by solvents and purified by chromatography [45]. AFB1 and other aflatoxins have been produced through this method by many chemical companies including Sigma-Aldrich, among others. While doing the purification, it is important to note that *Aspergillus flavus* produces only B aflatoxins and sometimes the mycotoxin cyclopiazonic acid (CPA), while *Aspergillus parasiticus* produces both B and G aflatoxins but not CPA. Various mutants of *Aspergillus flavus* have varying relative stability ratios of B2/B1 [45, 46]. Ada and Matcha [46] described a method for aflatoxin production by fermentation in which an *Aspergillus flavus* strain isolated from groundnut, referred to as *Aspergillus flavus* strain AJ, was used. The *Aspergillus flavus* strain AJ was found to be very stable and consistently yielded higher levels of aflatoxins, especially AFB1, after transfers [46]. In their preparation Ada and Matcha [46] used inoculums prepared by inoculating tubes (1.5 × 15 cm) of potato-dextrose-agar with spores of the AJ. This strain was used to produce an aflatoxin stain AJ2010. The potato-dextrose-agar gel was prepared by adding 20 g dextrose, 20 mg NaCl and 1 g of agar in 100 ml distilled in a conical flask, adjusting and maintaining the mixture at pH 7. The mixture was kept momentarily at 121°C in an autoclave and then platted in a laminar flow [46, 47]. The inoculated slants were then incubated for 7–21 days at 28°C after which the cultures had a heavy crop of green *conidia*, and the spores were scraped loose with a loop. The slants were shaken to give a uniform suspension of spores, and the spore suspension (0.5 ml) was used to inoculate each of 100 g of the substrate (groundnut), a fish feed. Fermentation which involved the growth of *A. flavus* on the feed (100 g) at high moisture levels to produce a pale green aflatoxin substrate was carried out by mixing 25 ml distilled water with 50 g of fish feed in an Erlenmeyer flask. The mixture was allowed to stand for 1 h with frequent shaking, and then the flasks were autoclaved at 15 psi for 15 min before cooling and inoculation, keeping the flasks at 28°C and blending on a shaker at 188 rev/min. The flasks were removed, and the feed was prevented from binding with

the produced mould mycelium by shaking vigorously. The product could be used for experiments directly or for extraction of a concentrate of aflatoxins using 80% methanol as explained by Nelson et al. [48] and Ada and Matcha [46, 48].

3. Analysis and identification of aflatoxin B1

Several sampling and analytical methods which include thin-layer chromatography (TLC), high-performance liquid chromatography (HLPC), mass spectrometry and enzyme-linked immunosorbent assay (ELISA), among others, have been used to analyse aflatoxin B1 in various contaminated foods [49]. According to the Food and Agriculture Organization, the worldwide maximum tolerated levels of aflatoxin B1 were reported to be in the range of 1–20 μg/kg in human foods and 5–50 μg/kg in dietary cattle feeds in 2003 [50]. Apart from these limits, the WHO, EU, USFDA and Kenya Bureau of Standards (KEBS) have set international and national maximum limits for a specific aflatoxin metabolite (e.g. AFB1) level, as well as a total concentration which involves the summation of concentrations of all detected metabolites (AFB1, AFB2, AFG1, AFG2 and AFM1) in a sample. It is therefore important to optimize and interpret standard procedures for extraction, detection and quantitation of aflatoxins in a sample. A review of the methods that have been used is presented in the following paragraphs.

Various researchers, including analysts, food specialists and health workers, have been involved in the analysis of aflatoxins including AFB1 in various materials including samples of human specimens, animal tissues, food, grains, cereals and legumes. Aflatoxins, AFB1 included, have been characterized by nuclear magnetic resonance (NMR) and liquid chromatography-mass spectrometry (LC–MS), and their mass spectral data are available in LC–MS libraries making it possible to screen samples rapidly. In addition, retention times and column flow-through patterns for aflatoxins combined with high-purity reference standards can be used in HPLC and other analytical techniques. Aflatoxins B1, B2, G1 and G2 have been determined quantitatively by HPLC with a fluorimetric detector using toluene as a mobile phase [51]. This method is applicable to food and feed extracts. Several AOAC official methods have been used to analyze AFB1 [1, 52]. These methods include ELISA, TLC and HPLC. TLC and fluorescence detection methods sometimes have reported high detection limits and are not used frequently nowadays for forensic purposes despite their popularity in the past. The methods for determination of aflatoxins in food samples and cereals for animal consumption can be validated as explained in the EC No. 882/2004 and EC No. 401/2006 methods, demonstrating their conformity with these methods, in terms of sensitivity, linearity, selectivity and precision [53].For mass spectral data, tandem mass spectrometry data containing a METLIN-tested metabolite database generated independently by the Scripps Center for Mass Spectrometry and Metabolics for identification of metabolites are available for reference in pdf. This product is available in Sigma-Aldrich. Other libraries are available for referencing including a Sigma-Aldrich database which presents HPLC Analysis of Aflatoxin Analogs on Ascentis® C18; a Sigma-Aldrich LC/MS/MS Analysis of μL Mycotoxins on Ascentis® Express Phenyl-Hexyl column and a Sigma-Aldrich UHPLC–MS/MS Analysis of μL Mycotoxins on Titan™ C18.

A high-performance liquid chromatographic method with online post-column photochemical derivatization and fluorimetric detection was used for simultaneous separation and quantitative determination of AFB1 and other metabolites in foodstuffs and feed material [53]. In one study, the chromatographic separation was accomplished by using a C18 column and analytes were eluted with an isocratic mobile phase consisting of water/methanol/acetonitrile [52]. In this method sample

preparation requires simple extraction of aflatoxins with a mixture of water and methanol followed by a clean-up and a chromatographic separation step by immunoaffinity column and then detection [53]. Efficient analysis of aflatoxins B1, B2, G1 and G2 has also been achieved by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, using a UV-absorbing ionic liquid matrix with addition of NaCl to obtain matrix-free mass spectra, which enhances sensitivity via Na^+ cationization [53]. Using ionic alpha-cyano-4-hydroxycinnamic acid (Et3N-alpha-CHCA) as the matrix, the matrix-free mass spectra in the m/z range of interest were acquired, and the B1, B2, G1 and G2 aflatoxins were readily detected with very low detection limits [53]. This technique is fast and requires minimal sample preparation (just mixing the liquid matrix with methanol extract), and no derivatization nor chromatographic separation is required. The method was reported to be suitable for rapid screening of aflatoxins including AFB1 in a wide array of major crops which are often subjected to huge world commercial trades such as peanuts, maize and rice, as well as to monitor bioterrorism threats by mycotoxin poisoning [53].

Analysis of aflatoxins in clinical laboratory procedures is also often done routinely by analyzing AFB1 in blood and urine. This has been done by HPLC with various columns and a fluorescence detector as reported by Seo et al. [54]. Aflatoxin B1 recoveries ranged from 33 to 95%, for spiked human serum samples following extraction using hexane chloroform, chloroform extraction and clean-up with pentane on a silica gel column or acetone-ferric gel-chloroform extraction and clean-up with pentane on a silica gel column [55]. This reverse phase HPLC procedure was also used successfully for aflatoxins and metabolites in animal tissues, in a process involving trifluoroacetic acid-catalyzed conversion of aflatoxin B1 to a fluorescent derivative B2 [55]. Human urine and methanol extracted from the kidney, liver, brain tissues and sputum have been analysed using competitive ELISA methods with immunoaffinity columns and fluorometry, with concentrations for urine, sputum and tissue biopsies found to range from 1.0 to 5.0 ppb, with negative control patients showing no detectable mycotoxins in their fluids or tissues [56]. This study confirmed that AFB1 and other aflatoxins can be detected in body fluids and human tissues from patients exposed to mycotoxin-producing moulds in the environment and indicated which tissues or body fluids are most likely to give positive results. A procedure involving salting-out-assisted liquid/liquid extraction for multi-mycotoxin biomarkers and subsequent analysis using high-performance liquid chromatography-tandem mass spectrometry, for pig urine, has also been reported [53].

Radioimmunoassays that can detect levels as low as 0.27 pmol (0.06 ng) of AFB1 have been used to analyse crude extracts of corn and peanut butter with just traces of aflatoxins, and in these foodstuffs, as little as 1 μg aflatoxin/kg has been measured by this technique [57]. Detection limits for radioimmunoassay techniques vary ranging from 1 up to 5 μg/kg in various matrices including corn, peanut butter, cottonseed products, groundnuts and groundnut products and other cereals [1].

Recently, a comprehensive technique involving detection and quantification of aflatoxins using an AflaTest method has been described by William and George [58] and Orony et al. [59]. In this method, the presence of aflatoxins was tested in a screening step by TLC using the solvents hexane, petroleum ether, chloroform, acetone and toluene (10:10:60:10:10), and fluorescent spots were checked under UV light [59]. An AflaTest affinity column is an immunoaffinity column bound with specific antibodies of aflatoxin. When a sample is passed through, the aflatoxins become bound to the antibodies in the column [58]. A volume of 1 ml of the extract was diluted with distilled water and mixed well before filtering through a glass microfiber filter, and an aliquot of the filtrate was pipetted and passed through the AflaTest affinity column [59]. The column was cleaned twice with distilled water to remove the immunoaffinity impurities, and then aflatoxins were eluted from the column with HPLC-grade

methanol and collected in a cuvette. A known volume of a developer solution (bromine solution in distilled water (5:45 vol/vol)) was added to the eluate, and then aflatoxin content was determined in the mixture using a fluorometer after a short period of 1 min. The fluorometer can have an inbuilt aflatoxin calibration standard, and it detects the intensity of the fluorescence which is determined by the amount of total aflatoxin present in the sample, and then a digital read out is obtained [59]. The limit of detection of the aflatoxins in this method was very low, about 0.05 μg/kg. Samples analysed using this method included fresh, smoked and grilled fish.

Wasike [60] used an ELISA method, which is recommended by the FAO for rapid screening of agricultural produce such as grains and involves several steps including the following: *coating* where the polystyrene plates are treated with a standard solution of either an antigen or antibody of the aflatoxin, *blocking* where unrelated protein-based solution is used to cover all the unbound sites on the plate, *detection* where enzyme-conjugated antibody or antigen binds specifically to the target antigen or antibody and *read out of results* in which the substrate (extract) is added and the signal produced by the enzyme-substrate reaction (binding) is measured [50]. The measurement can be done for total aflatoxin or single metabolite (e.g. AFB1), respectively, by UV–VIS using a calibration standard, prepared by pure analytical grade (>95% purity) AFB1 obtained from suppliers. Quantitation is based on absorbance readings (at 450 nm) versus concentrations of known standards. Several recommended quality assurance procedures were followed as described by [50]. A number of laboratories in developing countries including Kenya, where aflatoxin contamination is highly prevalent, have received training and funding to establish their own laboratories which are equipped with necessary instrumentation from the FAO to enable them to achieve rapid screening of samples using this ELISA method [50]. The FAO [50] procedure is simple, and for grains such as maize, 1 kg of the sample is weighed and milled using an electric grinder (mill). About 2 g of the ground sample is weighed into a screw-cap glass vial. This is then followed by addition of 10 ml methanol/distilled water (in the ratio of 70:30 v/v) and mixing for 10 min at room temperature using a shaker. The entire extract is filtered using a Whatman filter paper No. 1. Then 100 μl of filtrate is diluted with 600 μl distilled water of which 50 μl is employed as the substrate per well in the assay [50]. An aflatoxin test kit containing standard solutions of microtiter plate with 96 wells coated with capture antibodies; aflatoxin standard with ranges of concentrations of aflatoxin B1; a conjugate (6 ml)—peroxidase-conjugated aflatoxin B1—ready for use; anti-aflatoxin monoclonal antibodies (6 ml); Red Chromogen Pro (10 ml), a substrate/chromogen solution stained red and a 1 N sulphuric acid stop solution (14 ml) which converts the reactants from blue to yellow colour is provided [60]. A buffer salt (washing buffer pH 7.4) and distilled water and 70% methanol solution (70:30 vol/vol, methanol/distilled water), respectively, are prepared and made available for extraction of ground/homogenized material.

Nduti et al. [26] recently analysed aflatoxin B1 in cereals and other agricultural produce including sun-dried grains of maize and millet, maize flour and millet flour samples by PCR, a modified procedure similar to the ELISA methods reported by other researchers [50, 58, 59, 61]. The samples were transported immediately after sampling in cool boxes to an ISO 1705 accredited by Kenya Bureau of Standards laboratory and stored at −20°C until analysis was started. After grinding in a blender, known masses were weighed into disinfected beakers for extraction with a known volume of 70% methanol (in deionized water) by stirring. This was followed by filtering into a disinfected conical flask using Whatman filter paper No. 1. The residue on the filter paper was discarded and the filtrate preserved in the beaker for analysis. For analysis of aflatoxins, a known volume of a conjugate was introduced into the microwells using a micropipette, and then small aliquots of the filtrate were

added [26]. A sample of 20 ppb of aflatoxin was put into one of the microwells as a control. After, 100 μl of the sample plus conjugate mixture was transferred to antibody-coated microwells and the mixtures incubated for 15 min. The method of Leszczynka et al. [61] was modified by using a specific conjugate mixture, thus eliminating the need for wells pre-washed with phosphate buffer solution (PBS). The PBS cleans the unbound proteins but also reduces sensitivity at the enzyme reaction site [62]. After incubation, the contents of the microwells were discarded and the microwells washed at least five times with distilled water to remove the nontoxin reactants [26]. After draining the water, an aliquot of the substrate solution was put into each of the microwells before incubation for another 5 min. The free and peroxidise-combined aflatoxins compete for the sites with mouse antibodies that are immobilized on the plates. The reaction in this process results in a colour change from a clear to a blue colouration, whose intensity indicates the aflatoxin content. A deeper colour indicates more reaction and binding with the substrate and less aflatoxin concentration in the sample. To stop the reaction, an acidic stop solution was added, which resulted in colour changes from blue to yellow, depending on the aflatoxin levels [26]. The resultant solutions in the microwells were fed into a microtiter plate PCR reader where the optical density of each microwell was read using a 450 nm filter, and the amount of total aflatoxin present in each sample was determined quantitatively online and recorded on a computer [26].

The maximum levels (MLs) are established in various countries in Europe and the USA using various standard ELISA-based procedures [63]. For aflatoxin B1, the 5121AFB method and its kit provide a competitive enzyme immunoassay based on antibodies directed against anti-aflatoxin B1 [63]. The kit includes 96 wells 12 × 8 break-apart. The conjugate is aflatoxin-horseradish peroxidase. Rapid sample preparation procedures for cereals, rice, eggs, nut, honey, mashed fruits edible oils and feed are included in the kit manual. Antibody cross-reactivity includes aflatoxin B1 (100%), aflatoxin B2 (20%), aflatoxin G1 (17%) and aflatoxin G2 (4%). These standard procedures involve conjugate and standard/sample being pipetted into the wells and incubated for 1 h at 37°C. After washing, the ready-to-use substrate is added and incubated for 30 min at 20–25°C. The reaction is stopped and the absorbance read in a UV spectrophotometer at 450 nm. A EuroProxima software converts the measured optical density into concentration of the metabolite in the starting material. The assay limits of detections (LOD) (in ppb), calculated as Xn + 3SD as determined under optimal conditions, are cereals (0.5), rice (0.4), eggs (0.2), nuts (0.75), honey (0.2), mashed fruits (0.6), edible oils (1.0) and feed (1.0). The calibration standard concentrations ranged within 0, 0.0157, 0.0313, 0.0625, 0.125, 0.25 and 0.5 ng/ml [63].

Direct evidence for human exposure to AFs by ingestion or another route has been found in a number of countries by identifying AFs or their metabolites in human biological samples [46, 64]. Thus, it is becoming a significantly important issue for health of adults and people who are directly exposed to food contaminated with AFs [46, 64, 65]. Analyses of human specimen samples have to be done sometimes both for forensic and research purposes. In one analytical procedure [56], 100 mg of kidney sample was added to 1 ml tubes containing 1 ml 50% methanol before incubation for 5 min, until it completely dissolved. After, the suspensions were centrifuged at 10,000 rpm for 10 min and the upper layers (800 μl) collected into 2 ml glass tubes, before taking 5 μl for analysis using a UHPLC Q-Orbitrap, with triplicate measurements for each aliquot. Metabolites were separated in a UHPLC system (Dionex UltiMate 3000) equipped with a Waters column (Acquity BEH C18 1.7 μm, 2.1 × 50 mm) incubated at 40°C. The mobile phases were made up of water containing 0.1% formic acid and 2 mM ammonium formate (solvent a) and acetonitrile (solvent b), as explained [56]. The Q Exactive instrument, equipped with thermoelectrospray ionization in positive and negative switching modes, was utilized to detect the

aflatoxins in the above samples, and the system was calibrated and controlled by a software (Xcalibur 3.1 and Q Exactive Tune) [56]. The UHPLC Q-Orbitrap analysis can produce large amounts of raw data using TraceFinder software [56]. In addition, kidney tissue was isolated and fixed in 4% paraformaldehyde for 48 h, before paraffin embedding and sectioning using a microtome (Leica, Germany); and the sections were stained, and the histopathology was assessed under a light microscope (Olympus, Japan), with photographs being taken at 200× magnification, for confirmation of aflatoxin exposure [56]. Blood samples were centrifuged to collect serum (15 min at 3000 rpm and 4°C) for measurement of biochemical parameters, including creatinine, urea, uric acid, malondialdehyde, superoxide dismutase and total antioxidant capacity, which were undertaken using ELISA kits [56].

In another analytical method for AFB1, ELISA, TLC and HPLC were validated and used for identification of aflatoxin B1 (AFB1) in contaminated fish feed, media and fish serum samples [46, 48, 66–69, 122]. The analysis and identification of AFB1 was achieved using a DOA-ELISA test kit, followed by TLC with retention factors of 0.81, 0.79, 0.81 and 0.80 for AFB1-contaminated fish feed, media and serum samples, respectively, co-chromatographed with an AFB1 reference standard. HPLC results showed that the AFB1 levels in contaminated fish feed, media and serum samples were 2.6, 2.6 and 2.7 ng/ml, respectively. The concentrations of AFB1 were almost similar for all the three samples but slightly higher in the fish serum sample which had 2.7 ng/ml; and it was therefore concluded that because of its accuracy and sensitivity when compared with routine methods of AFB1 analysis, fish serum provides a sensitive specimen for AFB1 analysis in fish. This TLC-HPLC method was strongly recommended for monitoring AFB1 contamination in feed stuffs, especially in fisheries where the feed is under continuous exposure to moisture. The method is highly recommended in aquaculture and fisheries to screen the mycotoxins in fish feed as it gives a measure of bioaccumulation of these toxins in fish serum which can be correlated well with toxic effects on different environments like in vitro and in vivo to help in ensuring safety and measuring AFB1 tolerance. In one study [46], detailed methods for fermentation using an inoculated *Aspergillus flavus* strain isolated from groundnut to produce aflatoxins which were used to validate the analysis by TLC, HPLC and ELISA were presented.

Direct determination of urinary mycotoxins is a better approach to assess individual's exposure than the indirect estimation from average dietary intakes [70]. In a study by Fouad et al. [70], a new analytical method was developed and validated for simultaneous analysis of aflatoxins including AFB1 in urine based on ELISA. Like other ELISA methods so far described, the phenomenon of fluorescence quenching of an antibody by a specific ligand was applied in developing the technique for detection of mycotoxins, such as aflatoxin B1, ochratoxin A and zearalenone where loss of absorbance corresponds to inverse of concentration of aflatoxins [71].

Detecting aflatoxicosis in humans and animals is difficult due to variations in clinical signs and the presence of other factors such as suppression of the immune system caused by an infectious disease [72]. Of the two techniques most often used to detect levels of aflatoxins in humans, the first one involves measurement of the metabolite in urine (which however is only present for 24 h after exposure), and the second one involves measuring the level of aflatoxin-contaminated nuts, an AFB-albumin compound in the blood serum, providing information on exposure over weeks or months [72]. These biomarker measurements are important in investigating outbreaks where aflatoxin contamination is suspected. A variety of methods for detection of aflatoxins in food and feed that are highly specific, useful and practical have so far been discussed and are available for different needs. Methods are therefore available for different needs, ranging from techniques/methods for regulatory

control in official laboratories (such as high-performance liquid chromatography-mass spectrometry (HPLC–MS)) [73, 74] to rapid test kits for factories and grain silos such as enzyme-linked immunosorbent assay (ELISA) [50, 73]. Potential novel aflatoxin detection systems, based on emerging technologies, include dipstick kits, hyperspectral imaging, electronic noses, molecularly imprinted polymers and aptamer-based biosensors (small organic molecules that can bind specific target molecules). The latter technologies may have relevance in remote areas because of their stability, ease of production and use. Sampling procedures for aflatoxin monitoring in export and import produce are problematic because moulds and aflatoxins are not evenly distributed throughout bulk shipments and batches of stored grain, and appropriate sampling is critical to get a representative result. Protocols for sampling procedures have been developed, in particular in the context of regulatory control. For instance, in setting maximum levels for aflatoxins, the Codex Alimentarius Commission has specified the protocols to be used for peanuts, almonds, Brazil nuts, hazelnuts, dried figs and pistachios intended for further processing and for ready-to-eat products [75]. The FAO of the United Nations [50] has developed a mycotoxin sampling tool which is available online. Recommended sampling methods are difficult to achieve, especially for subsistence farmers in rural areas who do not produce enough grain to spare the quantities needed for accurate testing. Thus, there is a need to develop rapid, low-cost, low-technology and accurate detection methods for aflatoxins to improve surveillance and control in rural areas. Organizations, such as the Partnership for Aflatoxin Control in Africa and the World Food Programme, are addressing these issues. The World Food Programme has instituted a Purchase-for-Progress Programme to ensure grain quality by creating a blue box, which contains test kits for grain quality, including aflatoxins [76]. Some of the problems encountered in sampling in Kenya have been discussed [76].

The main concern in aflatoxins exposure is that once they are formed, they are heat stable so that neither cooking nor freezing can destroy them completely and they therefore remain in food indefinitely and can cause sublethal effects in the body of humans and animals [15–17, 26, 29, 36, 38, 39]. When given a sample of food or a specimen such as human milk for a forensic test, it is possible to predict which particular aflatoxin is suspected depending on the type of food, feed or specimen. There is potential increase in consumers' health risks if higher levels of aflatoxins are permitted for various crops and other products. For example, increasing the current MLs from 4 μg/kg total aflatoxin to say 8 or 10 μg/kg for nuts such as cashew nuts, almonds and hazelnuts would have minor effects on the estimated dietary exposure, on the risk of cancer and the calculated margin of exposure, but due to carcinogenicity and genotoxicity limits, the MLs should be kept very low. The development of new methods for detecting and quantifying traces of aflatoxins and their metabolites in various matrices in future will influence not only the MLs but also reduce their lethality following human exposure.

Highlights on how changes in temperature, humidity, rainfall and carbon dioxide production due to climate change impact on fungal behaviour and consequently mycotoxin production have been investigated by researchers in Europe. Climate change has been reported as a driver for emerging food and feed safety issues worldwide, and the expected impact on the presence of mycotoxins in food and feed is of great concern [77]. AFB1 has the highest acute and chronic toxicity of all mycotoxins; hence, the maximal concentration in agricultural food and feed products and their commodities is regulated worldwide [77]. In this regard, the methods of analysis and detection, the structures and characteristics of aflatoxins and modelling of their maximum levels in various produce are expected to change in the future with changes in climate

4. Conditions for production of aflatoxin B1

The different species of *Aspergillus* require different conditions for optimal growth. The optimal temperature for *A. flavus* and *A. parasiticus* fungal growth and aflatoxin production is 35 and 33°C, respectively, and neither *Aspergillus* species produces aflatoxins when developed below 7.5 nor above 40°C [78]. Some researchers have reported ideal temperatures between 20 and 35°C and ideal relative humidity of more than 85% as optimal for growth of *Aspergillus* species and aflatoxin production [79]. According to other reports, usually the most important variables are the moisture content of the substrate and the relative humidity of the surroundings [80]. The moulds live in soil, surviving off dead plant and animal matter, but spread through the air via airborne *conidia* [26]. The moulds are often found in the outdoor and indoor air, in water, on food items, and in dust [81]. *Aspergillus flavus* and *Aspergillus parasiticus* are closely related and grow as saprophytes on plant debris of many crop plants left on and in the soil. They belong to the *Trichocomaceae* family and have a worldwide distribution but are commonly found in tropical climates with extreme ranges of rainfall, temperature and humidity [81]. Members of *Aspergillus* genus are characterized by production of nonseptate conidiophore which is quite distinct from septate hyphae; they are swollen at the tip to form a vesicle in which specialized spore-producing cells (phialides or sterigmata) are found. These specialized cells are either uniseriate or are short growths of biseriate metulae [81]. Colonies of *Aspergillus flavus* are green-yellow to yellow-green or green on Czapek's agar. They normally have biseriate sterigmata and reddish brown sclerotia. The conidia are coarse roughened and vary in size and are oval to spherical in shape. Colonies of *A. parasiticus* are dark green on Czapek's agar and remain green with age, and their sterigmata are uniseriate and usually have no sclerotia. Unlike *A. flavus*, the colonies of *A. parasiticus* are uniform in size and shape [81].

Improper farming practices have led to an increase in mould growth and aflatoxin contamination in crops and animals. Improper feeding habits such as feeding animals with spoilt maize, feeding mouldy human food to animals and blending of mouldy cattle feed with a fresh batch are some of the bad practices found in Kenya [26]. In common agricultural practice the rotten maize cobs are separated from the good maize cobs which are later shelled and milled. The rotten maize grains are used, by mixing one bag of clean grains and two bags of rotten grains, to make animal feeds [25]. This practice of dilution does not drastically reduce the amount of aflatoxin contamination in animal feeds, and hence, commercial feeds in Kenya have been found to be contaminated with aflatoxin B1 and milk with aflatoxin M1 [82]. The eastern part of Kenya has been found to have more cases of historical occurrences of aflatoxin contamination, while the central and western parts have shown increased risk of aflatoxin contamination [83]. Transferring of seeds, crops, animal feeds and animals from one region to another can also introduce *Aspergillus* in areas where it not was found originally.

Aflatoxins often occur in crops in the field before harvest and are usually associated with drought stress [79]. Poor storage conditions, especially during rainy seasons, can increase concentration of aflatoxins in produce [26]. They occur mainly in hot and humid regions where high temperature and humidity are optimal for mould's growth and toxin production [26]. The growth of fungi is caused by a number of factors which provide an ideal environment that promotes the growth [83]. The conditions that must all be prevailing for fungal growth to occur in Kenya include relative humidity above 70%, temperatures of over 30°C for a period of a few days to a week and stress to the affected plant, such as drought, flood or insect infestation. Furthermore, there must be high moisture content of crop (20% or higher) [24]. The prevailing climatic conditions in Kenya, which include drought, erratic rainfall,

high temperatures ranging between 20 and 35°C and high humidity (40–89%), provide a favourable environment for growth of mould and production of aflatoxins [84]. Mould usually does not grow in properly dried and stored foods, and therefore efficient drying of commodities and maintenance of the dry state, or proper storage, are an effective measure against mould growth and production of mycotoxins [25]. Therefore, to minimize the health risk from mycotoxins, people are advised to inspect whole grains (especially corn, sorghum, wheat, rice), dried figs and nuts such as peanuts, pistachio, almond, walnut, coconut, Brazil nuts and hazelnuts, which are all regularly contaminated with aflatoxins for evidence of mould, and discard any that look mouldy, discoloured or shrivelled [11]. They are also required to avoid damage of grains before and during drying and in storage, as damaged grain is more prone to invasion of moulds and therefore mycotoxin contamination [24].

Researchers have reported on *Aspergillus* growth in maize and millet and contamination of agricultural soils in Kenya and the conditions and mechanisms that encourage their growth, which include (i) contamination of grains when they come into contact with *Aspergillus* fungal spores in soils, (ii) transfer of spores onto maize cobs when still on the plant by wind, (iii) high tropical temperatures existing in maize and millet growing regions, (iv) changes in seasons from wet to dry with hot and humid or damp conditions providing most favourable conditions, (v) insect pest damage causing 'open wounds' on seeds/grains, (vi) mistiming of ideal harvest periods, (vii) failure to separate damaged cobs from good cobs and (viii) intensional mixing of bad grains with good grains with intention of lowering aflatoxin levels [26, 60]. The lack of adherence to handling procedures such as adequate drying period, maintaining required moisture levels, the lack of optimal ventilation and temperature during storage, failure by national cereal board to purchase the grains from farmers on time and failure of the board to follow regulations such as performing analysis on moisture and aflatoxin presence in the products to ensure levels conform the required maximum level standards during their purchase [76] have also been cited as factors which encourage moulding and aflatoxin contamination in maize. The growth of moulds is also dependent on the type of crops and their nutrient content [26]. Mould growth in maize is very common, especially in warm humid climates, because it is a good substrate for mould growth, especially those species that produce aflatoxins [78, 79]. The high carbohydrate content provides the two carbon precursors for mycotoxin synthesis [79]. Other cereals such as millet, rice and sorghum and legumes also face the same threat from aflatoxin production and contamination in Kenya.

Biodegradation and metabolism of AFB1 can also generate aflatoxin metabolites in animals, human and the environment. Aflatoxin M1 (AFM1) is a product of aflatoxin B1 (AFB1) metabolism and is found in milk in areas of high aflatoxin exposure [26]. Subsequently humans may be exposed to this aflatoxin through milk and milk products, including breast milk, especially in areas where poor-quality grain is used for animal feed. The principal hydroxylated AFB1 metabolite present in most milk of cows fed with a diet contaminated with AFB1 is aflatoxin M1. Aflatoxin M1 is usually excreted after 12 h in milk and urine when animal feed contaminated with aflatoxin is administered to the animals [22]. The hydroxylated metabolite is formed as a result of biotransformation of AFB1 and AFB2 by hepatic microsomal mixed-function oxidase (MFO) system. Improper farming practices described earlier have led to an increase in risk of contamination. Commercial feeds have been found to be contaminated with aflatoxin B1 and milk with aflatoxin M1 [82]. Metabolites B2 and G2 have also been produced and detected in soil through biodegradation processes [24]. Food crops can become contaminated both before and after harvesting [24]. Preharvest contamination with aflatoxins is mainly common to grains such as maize, millet, cottonseed, peanuts and tree nuts. Postharvest contamination can be found in a variety of other crops such as coffee, rice and spices. Improper storage

under conditions that favour mould growth can lead to levels of contamination much higher than those found in the field [22]. Apart from grains, postharvest production of *Aspergillus* species has resulted in aflatoxin production in other food types such as sun-dried fish in Kenya and other African countries due to poor handling and storage conditions [59, 85].

While toxicity of aflatoxin metabolites are now well recognized, it is not often known that *Aspergillus flavus* fungi itself also exerts pathogenic effects through aspergillosis or infection with the mould, which largely occurs in the lungs, skin and other organs of immune-compromised patients [65].

5. Toxicity and metabolism of aflatoxin B1

Aflatoxins are very toxic to mammals with the LD_{50} (oral, rat) being 4.8 mg/kg body weight for AFB1 reported and also to domestic animals with AFB1 LD_{50} (oral) values of 0.5 (dogs), 0.62 (pigs), 2 (guinea pigs) and 6.3 mg/kg (chicken) [86, 87]. They are known human carcinogens, and there is sufficient evidence for carcinogenicity of AFB1 in animals and human based on in vivo and in vitro studies that have been done [86, 87]. AFB1 has also been shown to be a potent mutagen and covalently binds to DNA, RNA and proteins in the liver. It is activated in the liver cells and induces principally G to T mutations [88]. DNA damage response which acts as an antitumor mechanism against genotoxic agents has confirmed that AFB1 is genotoxic. Genotoxicity studies of AFB1 on human embryo and adult liver cells in vitro have demonstrated the order of toxicity as B1 > G1 > G2 > B2 [86, 87]. Although AFB1 is a potent liver carcinogen in animals, in epidemiological studies done in Africa, it has been difficult to ascribe the incidence of human liver cancers solely to AFB1 because of concurrent exposure to other potentially causative agents (e.g. liver parasitism, hepatitis B virus, other mycotoxins as well as other carcinogenic environmental and food contaminants) that may be enhancing factors for liver damage and replication [89]. However, AFB1 binding to DNA and consequent interference with host genomes have been established and confirmed by mechanistic and inhibition studies [90]. Previously, some epidemiological studies were conducted on cancer patients aimed at evaluating the effects of AFB1 and AFM1 exposure on cancer cells in order to verify the correlation between toxin exposure and cancer cell proliferation and invasion [64].

The International Agency for Research on Cancer (IARC) has classified AFB1 and AFM1 as human carcinogens belonging to Group 1 and Group 2B, respectively, with formation of DNA adducts identified [25, 45]. Aflatoxins play a causative role in 5–28% of hepatocellular carcinoma (HCC) worldwide [91]. Marchese et al. [64] have recently reviewed the chemistry and metabolism of AFB1 and AFM1 and their involvement in cancer development. They summarized the activation pathways of AFB1 and AFM1 and stated that AFB1 epoxidation is the key step in the genotoxic process and thus in the carcinogenesis, whereby the high affinity of the epoxide intermediate for purine bases of DNA was shown to lead to formation of AFB1-N7-Gua adduct that promoted mutations in nucleotide sequence. AFB1 is mainly metabolized in the liver upon action of the microsomal mixed-function oxidase (MFO) enzymes belonging to the superfamily of CYP450. It is converted into the reactive 8,9-epoxide in a process mediated by these oxidases. The epoxide exists as two stereoisomers, exo and endo, with the former being the toxic species responsible for AFB1 genotoxicity [92]. The exo-8,9-epoxide has a high binding affinity towards DNA, forming the 8,9-dihydro-8-(N7-guanyl)-9-hydroxy-AFB1 (AFB1-N7-Gua) adduct, thus leading to DNA mutations [64]. Epoxide formation is also involved in other metabolic pathways, including (i) conjugation with glutathione (GSH) catalyzed by glutathione-S-transferase (GST) with subsequent excretion as AFB-mercapturate, a pathway which is vital for the detoxification

of AFB1 as a carcinogen, even though a depletion of GHS was also reported to lead to high levels of reactive oxygen species (ROS) causing oxidative damage [93]; (ii) enzymatic and non-enzymatic conversion to AFB1-8,9-dihydrodiol, which can further be converted into a dialdehyde form, and an aflatoxin dialdehyde subsequently which can get excreted through urine as dialcohol upon action of aflatoxin aldehyde reductase (AFAR) or can bind proteins, like albumin [92] and (iii) binding to other macromolecules like proteins or RNA, causing inhibition of proteins, DNA and RNA synthesis and dysregulation of normal cellular functions [94]. Microsomal biotransformation of AFB1 also includes hydroxylation of the toxin, leading to the formation of more polar and less toxic metabolites, including mainly AFM1 and aflatoxin Q1 (AFQ1). Different studies tried to assess the role of the CYP450 enzymes which are responsible for detoxification and formation of carcinogenic metabolites. CYP1A2 and CYP3A4 strains were found to be capable of activating AFB1 and the most active isoenzymes of the CYP450 family to do this [28]. It has been reported that CYP3A4 is responsible for the formation of AFB1-exo-8,9-epoxide and trace amounts of AFQ1, whereas CYP1A2 leads to both exo- and endo-8,9-epoxide and eventually to the hydroxylated AFM1 metabolite [27]. The other two isoenzymes that use AFB1 as a substrate to a minor extent are CYP3A7, expressed in the human foetal liver, and CYP3A5 [27]. Other mechanisms of AFB1 toxicity include formation of intracellular reactive oxygen species which cause oxidative damage, resulting in AFB1 inducing cytotoxicity; and studies have demonstrated oxidative stress-induced toxic changes in the liver related to AFB1 toxicity [90, 95] oxidative stress-induced apoptosis through a mitochondrial signal pathway which has been reported [96]. AFB1 has caused oxidative and nitrosative hepatoxicity in rat and chick hepatocytes [90]. The predominant mutation caused by AFB1-N7-Gua adduct has been identified, and the sites of mutation and selectivity towards guanine bases have been elucidated [64]. These mutation studies have confirmed the links with a great number of epidemiological data on hepatocellular carcinoma (HCC) patients from regions of high aflatoxin exposure, strengthening the association between HCC incidence and aflatoxin exposure [97]. Research on human exposure to AFB1 through diet and analysis of liver and plasma metabolites have demonstrated hepatocarcinogenesis, with plasma concentrations showing that absorption and metabolism of AFB1 are rapid in human.

It has been noted that AFM1 is primarily considered a detoxification product of AFB1 metabolism, showing only 10% of mutagenicity compared to its precursor [92], and its metabolic fate is similar to that of AFB1, with the difference that AFM1 presents a poorer substrate for epoxidation, thus explaining the differences in genotoxic potencies. It has also been reported that CYP450 activation is not required for AFM1 to exert cytotoxic effects [92]. Apart from the principal biotransformation pathway involving CYP450, other activation mechanisms have been reported for aflatoxins. In fact, epoxidation catalyzed by prostaglandin H (PGH) synthase has been described by Battista et al. [98], whereas Weng et al. [99] have recently reported a mechanism in which lipid peroxidase (LPO) is the main enzyme responsible for AFB1-induced carcinogenesis, triggered by production of cyclic-methyl-hydroxy-1 and N2-propano-dG (meth-OH-PdG) adduct and/or inhibition of DNA repair.

Aflatoxicosis

Human intoxication by aflatoxins may occur via contact, ingestion and inhalation; and they can affect the liver, kidney, stomach and lungs, salivary glands, colon and skin [91]. Once ingestion of aflatoxin B1 has taken place, the gastrointestinal tract rapidly absorbs it with other aflatoxins, and the circulatory system transports them to the liver [100]. Approximately 1–3% of the ingested aflatoxins irreversibly bind to proteins and DNA bases to form adducts such as aflatoxin B1-lysine in albumin [101]. Disruption of protein and DNA bases in hepatocytes disrupts their functions and

causes liver toxicity [101]. This results into chronic exposure which is defined as the ingestion of very small doses of aflatoxins in a long period of time [101]. Ingestion of higher doses of aflatoxins can result in what is called acute aflatoxicosis [100]. The order of potency for acute and chronic toxicity is B1 > G1 > B2 > G2 [20]. AFB1 may not itself be toxic, but it is metabolized to produce more toxic metabolites, and its subsequent metabolism determines both the acute and chronic toxicity.

Bankole and Adebanjo [11] have defined aflatoxicosis as poisoning which results from ingestion of aflatoxins in contaminated foods in human and feeds in animals and manifests as chronic or acute aflatoxicosis. The term is therefore not restricted to human poisoning only but can be used to describe aflatoxin poisoning in other organisms including domestic animals, birds, fish and other organisms. Chronic aflatoxicosis results from ingestion of low to moderate levels of aflatoxins. Chronic dietary exposure to aflatoxins is a major factor for hepatocellular carcinoma [11]. Common subclinical symptoms are seen through impaired food conversion and slow rate of growth with or without production of an overt aflatoxin syndrome and liver cancer [11]. Ingestion of higher doses of aflatoxin can result in an acute aflatoxicosis which manifests as hepatotoxicity with symptoms of liver damage, hemorrhage and alteration of food digestion or, in severe cases, liver failure and death (which occurs in 25% of cases of acute poisoning) [81]. No animal species is resistant to the acute toxic effects of aflatoxins [11]. The biological effects of aflatoxin can be grouped into four general categories: acute and chronic liver damage, reduced growth rate, impairment of immunologic and innate defense mechanisms and carcinogenic and teratogenic effects, respectively, and different animal species respond differently. Aflatoxicosis can be influenced by environmental factors as well as by levels ingested, duration of exposure, age health, nutritional status and diet [81]. Aflatoxin B1 is a very potent carcinogen in many species including primates, birds, fish and rodents. In each species, the liver is the primary target organ of aflatoxin toxicity and carcinogenicity in acute injury [81].

Early symptoms of hepatoxicity from aflatoxicosis can manifest as anorexia, malaise and low-grade fever, which can progress to potentially lethal acute hepatitis with vomiting, abdominal pain, hepatitis and death [25]. Symptoms of AFB1 also include yellow eyes, swollen legs, vomiting, abdominal pain and bleeding. The health impact of aflatoxin exposure in animals mainly depends on dosage and response to the epidemic, and low dosages produce nutritional interference and immunological suppression, while high doses lead to acute illness and death [81]. Aflatoxins have been detected in the blood of pregnant women, umbilical cord blood and breast milk in African countries, with significant seasonal variations [24]. Levels of aflatoxins detected in the umbilical cord blood at birth are among the highest levels ever recorded in human tissues and fluids [24], and therefore mother-to-child transfer impacts are expected to be significant. Aflatoxins have been suggested as an aetiological factor in encephalopathy and fatty tissue degeneration of viscera, similar to Reye syndrome, which is common in countries with a hot and humid climate [101], an indication that exposure can lead to symptoms such as memory loss and dementia. Aflatoxins have been found in blood during the acute phase of the disease and in the liver of affected children [24]. In recent studies, aflatoxins have been found in the brains and lungs of children who have died from kwashiorkor and those who had died from various other diseases [21].

Outbreaks of acute aflatoxicosis from highly contaminated food have been documented in Kenya, India and Thailand [104]. In April 2004, an outbreak of an acute hepatotoxicity was identified among people living in Makueni, Kitui, Machakos and Thika Counties, and epidemiological investigation determined that the outbreak was as a result of aflatoxin poisoning from ingestion of contaminated maize [105]. In July 2004, 317 cases and 150 deaths had occurred, making this one of the largest

Year	Those affected	Numbers affected	Sources of toxin	Observed effects
1977	Poultry and dogs	Unspecified	Contaminated maize	Death
1981	Human	12	Contaminated maize	Death
1984/1985	Human	Unspecified	Contaminated maize	Death
1988	Human	3	Contaminated maize	Death and acute symptoms
2001	Human	29	Contaminated maize	16 deaths and acute symptoms
2002	Poultry and dogs	Large numbers	Contaminated maize	Death
2003	Human	6	Contaminated maize	6 deaths
2004	Human	331 (500*)	Contaminated maize	125 deaths and acute symptoms
2005	Human	75	Contaminated maize	32 deaths and acute symptoms
2006	Human	20	Contaminated maize	10 deaths and acute symptoms
2007	Human	4	Contaminated maize	2 deaths and acute symptoms
2008	Human	5	Contaminated maize	2 deaths and acute symptoms
2010	Human and dogs	Unspecified	Contaminated maize	Unconfirmed dog deaths; drop in prices

*Source of Information: Nduti [102].*Ngindu et al. [103].*

Table 2.
Aflatoxicosis cases in Kenya since 1977.

and most severe outbreaks of acute aflatoxicosis documented worldwide [106]. In 1981, an outbreak of aflatoxicosis from contaminated maize occurred in Makueni County and other parts of Kenya which reported 500 acute illnesses and 200 deaths [103]. In both 1981 and 2004, drought and food shortages were followed by unreasonable rains during harvest which probably favoured the growth of aflatoxigenic *Aspergillus* in household maize [107]. From the above cases, it is clear that aflatoxin food poisoning is a common phenomenon in eastern parts of Kenya and occurs on cereals commonly used by many communities as staple food. These cereals can be stored in processed and non-processed form. A summary of aflatoxicosis occurrences in Kenya is presented in **Table 2**.

6. Sources of AFB1 and exposure in Kenya

Dietary exposure varies greatly from country to country, and estimates of dietary exposure indicate clear differences between developed and developing countries [25]. In developed countries, mean aflatoxin dietary exposures are generally less than 1 ng/kg body weight per day, compared with some sub-Saharan African countries where mean exposure exceeds 100 ng/kg body weight [24]. The Center for Disease

Control and Prevention [108] has estimated that 4.5 billion people are exposed to aflatoxins worldwide, with the risks varying from country to country. In other reports, aflatoxin exposure in Africa ranged from 10 to 180 ng/kg body weight/day, while exposures in Europe and North America ranged from 0 to 4 and from 0.26 to 1, respectively [108]. A study done in Kenya has shown that populations from all economic strata have aflatoxin exposure [22]. The level of aflatoxin B1—the most toxic of the aflatoxins—in blood serum in individuals was found to be similar across the rich and poor, with the highest burden among the middle wealth quintile [22]. Climate changes have been reported to play a major role and would likely lead to increased occurrences of aflatoxins and other mycotoxins (and possibly their increased co-occurrence) in Kenya and other countries [22]. It has been reported that the tropical and subtropical regions of the world including sub-Saharan Africa and parts of Southern Asia are highly likely to continue experiencing aflatoxin-related contamination issues due to high temperature and humidity conditions, particularly damp conditions during the rainy seasons, and drought being experienced in these countries as these conditions increase crop susceptibility to aflatoxin contamination [25]. In another study, it was found that there was a low awareness and understanding of the dangers of mycotoxins in food and certain practices among farmers in Kenya could therefore increase the risk for exposure [76]. Gender analysis revealed that groups having knowledge were not always responsible for risk mitigation [83]. In a study conducted in the major farming regions in Kenya, it was found that 67% of the urban smallholder dairy farmers had no knowledge that milk could be contaminated with aflatoxin M1 and none knew how they could mitigate against this exposure [24, 109].

Bankole and Adebanjo [11] mapped Kenya into aflatoxicosis risky areas taking into consideration humidity, temperature, rainfall, dairy cattle density, feed resources, farming systems and consumption of maize and milk. The eastern parts of the country had more cases of historical occurrences of aflatoxin contamination, while the central and western parts showed increased risk of aflatoxin contamination [83]. In Kenya AFB1 and other metabolites have been analysed and detected in animal commercial feeds, grains, flour and cooked diets. Among researchers, aflatoxin analysis in human and cattle feed is one of the most common research topics especially by graduate students in the national universities, although research into its human health impacts has received less attention. In a study done in 2008, it was reported that most people in Kenya were exposed to low-level doses of a wide spectrum of fungal poisoning through regular consumption of cereals such as maize and cereal products [76]. For example, an average Kenyan eats maize products at the rate of 0.4 kg/person/day such that even the lowest amount of exposure can result in a cumulative exposure likely to cause health effects [76]. Maize is the staple food (accounting for more than 75% total cereal area) and is mainly grown by small holder farmers who together with their families account for 70% of the Kenyan population [76].

In a survey done in 2001, samples of agricultural produce including grains and flour obtained from ordinary grocery stores, kiosks, supermarkets and open-air markets in Nairobi and other towns in Kenya were found to be contaminated with moulds that produce aflatoxins among other mycotoxins [26]. Recently, the mean concentration levels of aflatoxins in dry maize grains in Kenya, as analysed by ELISA method, range from 2.51 to 17.4 ppb (dry weight) in samples taken from Western, Nairobi and Eastern provinces of Kenya [26, 60, 110]. Analysis of sun-dried maize, millet, flour and fish samples from different regions in Kenya found that, in general, there are aflatoxins including AFB1 in these products, even though at lower concentrations compared with standard maximum allowed levels by the WHO, FAO, EU and KEBS [26, 39, 60, 109, 110]. Wasike [60] determined total aflatoxin levels in randomly sampled maize grains from Bungoma using ELISA method

and found 2.51–3.56 ppb of total aflatoxins (based on dry weight) and concluded that there was no significant variation ($p < 0.05$) with site. He also reported lack of awareness among farmers on aflatoxins in the areas where samples were taken from and listed harvesting, drying, storage methods and prevailing rainy weather during harvesting as main factors that influenced the production of aflatoxins [60]. Okech [110] used solvent extraction and LC–MS to analyse branded (milled and packaged by commercial Millers) flour samples taken from supermarkets and unbranded (milled by traditional posho mills, packed in sacks and weighed according to customer needs in open markets) flour samples obtained from various open markets in Nairobi, Thika and Machakos. He found AFB1 in 67% of the unbranded flour samples with mean concentrations ranging from 1.07 to 8.89 ppb. About 33% of the samples from Kiambu showed aflatoxin levels with one sample having 8.89 ppb which was above the KEBS and Codex maximum level limit of 5 ppb, while 16.7% of the samples from Nairobi and Machakos had aflatoxins levels but were lower than the 5 ppb limit [110]. One sample of unbranded maize flour from Machakos contained AFG2 which was detected at a mean concentration of 6.02 ppb which was above the 5 ppb limit [110]. In terms of total aflatoxins, 22.2% of the samples of unbranded maize flour had aflatoxins but were below the 10 ppb KEBS and Codex maximum level limit [110]. There were no aflatoxins (all were below detection limit) in all samples of the branded flour samples which showed that commercial maize milling process in Kenya, which involves removal of unsuitable grains, dehulling, and removal of bran, lowers risks of aflatoxin exposure in human in Kenya [26, 110]. It was concluded that the levels of AFB1 were lower after commercial milling with concentrations in unbranded maize flour being much lower than corresponding dried grains [110]. Nduti et al. [26] analysed dried maize grains and flour samples taken from Western, Eastern and Nairobi regions of Kenya by ELISA and found significant variations ($p < 0.05$) in the three regions, with mean total aflatoxin level in grains ranging from 7.95 ± 1.57 ppb (Nairobi samples) to 22.54 ± 4.94 ppb (eastern samples), which were higher than the 10 ppb KEBS and Codex maximum limit and therefore a major source of concern. No significant difference in aflatoxins levels with site in flour was found, and the total aflatoxins levels were detected but were below the 10 ppb limit. Nduti et al. [26] found maize grains to be contaminated with aflatoxins (including AFB1) in samples from Nairobi and Eastern Kenya detecting aflatoxins in all samples with levels higher than the Codex and KEBS maximum limit of 10 ppb usage. The variations with site were insignificant ($p > 0.005$), and slight differences in mean concentration levels were attributed to differences in weather such as wind, temperature, insect damage of produce and storage and handling [26]. However, in maize flour which is the staple food for most of the population, the mean total level was slightly >5 ppb which was lower than the WHO level. In this study, aflatoxin contamination was confirmed by the presence of AFM1 in urine of the population [26, 35]. Nduti et al. [26] proposed that sorting, cleaning, bran removal and the use of chemical and biological agents to reduce the levels may have influenced lower concentrations in flour than maize grains. The results of Nduti et al. [26] suggested that cooked mixture of maize and beans (traditionally known as *githeri*) as the most likely source of human exposure of AFB1 in humans in the Eastern province of Kenya.

Recently, Orony et al. [59] reported mean total aflatoxins ranging from 0.33 to 1.58 ppb (wet weight) in sun-dried dagaa fish (*Rastrineobola argentea*) obtained from markets located along the Lake Victoria, caused by contamination due to poor handling and storage conditions. They estimated a daily intake of total aflatoxins of 0.0079 μg/kg/day during the rainy season when sun-drying is not effective. The risks involved in consumption of this fish in Kenya were found to be lower when considering the recommended maximum limits [50]. However, aflatoxins are remarkably

potent, often causing disease even when ingested in minute quantities. They are accumulative, resistant to degradation and also heat resistant implying that ingestion of minute amounts still poses risks. The generation of aflatoxins in processed dagaa was explained by the fact that the samples were collected from the markets during the rainy season in July, when drying was incomplete; hence, the sun-dried dagaa were packed in plastic sacks when they were not completely dry resulting in the growth of moulds [59]. The samples which were dried on a rack with some aeration were found to have lower levels of aflatoxin [59]. In Kenya, aflatoxin contamination was reported to be less common in foods during dry seasons as the drying process is more efficient during that period [50]. Incomplete drying condition has been associated with production of aflatoxins in previous studies [85]. Studies done in Nigeria by Bukola et al. [111] on smoked-dried fishes sold in the markets revealed that aflatoxins B1 (AFB1) and G1 were present in the samples at concentrations between 1.5–8.1 and 1.8–4.5 μg/kg, respectively. These reported cases of aflatoxin contamination in staple foods such as maize flour and fish in Kenya indicate that a very large proportion of the population in Kenya is potentially exposed to sublethal concentrations of aflatoxins including AFB1 in their diet. Previous studies conducted in Uganda, Swaziland, Thailand and Kenya have shown positive correlations between levels of aflatoxin contamination in market food samples and cooked food samples with incidences of hepatocellular cancer and mortalities [25]. However, research on biomarkers to quantify aflatoxin exposure in individuals have still not yet determined to link aflatoxin exposure with cancer risk in Kenya [4, 59].

There have been reported cases of aflatoxin outbreaks in Kenya which have led to severe poisoning in school children and adults fed on maize products, some of the products being donations by WHO food programmes for the school feeding programme [112, 113, 123]. These outbreaks of aflatoxin prevalence and aflatoxicosis have been blamed on the lack of regulations and control measures including lack of adherence to handling procedures such as drying period, maintaining required moisture levels, removal of damaged grains, lack of optimal ventilation and temperature during storage, prevention of insect damage which encourages moulding, failure by the national grain cereal companies to purchase the grains from farmers on time and failure to perform routine analysis of moisture and aflatoxin presence in the produce before milling [76]. It has been reported that the most critical interval of drying maize in Kenya is from when it starts drying up, down to approximately 20% moisture, and during this interval moulds occur more easily than any other period [26]. This period can be very long, ranging from 28 to 58 days, respectively, when traditional storage methods are adopted [26], during which, grains are subjected to extreme fluctuations in weather such as rainfall. In sub-Saharan Africa, weather is critical in addition to the prevalence of the S-strain of *Aspergillus flavus* which is more toxic as it produces more aflatoxins than any other strain of *A. flavus*. Nontoxic L-strain of *A. flavus* has been used to competitively exclude the producers through propagation during crop infection and thereby limit contamination in some countries such as the USA [76]. These highly competitive atoxigenic strains of *A. flavus* are applied to soil after which they spread to the crop, excluding the toxic strains [76]. There are about eight nontoxin-producing strains of *A. flavus*, which are capable of radically reducing aflatoxin in maize by 99% [76] and have been tried in Nigeria.

In Kenya, researchers at the Kenya Agricultural and Livestock Research Organization developed and manufactured a product called Aflasafe KE01 to fight aflatoxins in 2016 although this product has not yet trickled down significantly to the small-scale farmer. Aflasafe KE01 consists of four friendly strains of *A. flavus* which do not produce aflatoxins as active ingredient with sterilized sorghum and a binder making up 97% of the solid formulation which is applied preharvest by

broadcast during plant growth in the field (KALRO website, www.kalro.org). A single application is required in each cropping season to protect maize. The product has achieved between 80 and 99% reduction of aflatoxins in maize and groundnuts at harvest and in storage. Since soil is habitat for aflatoxin-producing fungi, contamination while the crop is still in the field or later during storage and processing is irreversible. Although Aflasafe KE01 reduces contamination in the field, it can only be effective if adopted alongside other safe standard procedures such as proper handling and storage of produce. However, the use of such techniques in Kenya has still not been felt nationally even though training of experts and building capability in Kenya for aflatoxin control have been very effective [76]. It has been recommended that contaminated maize and other grains such as millet be sold to ethanol producing factories since aflatoxins do not appear in the distilled alcohol [114], instead of using them to produce animal feed which makes the situation worse. Such developed country approaches for managing aflatoxin menace which include the use of food additives to make grain safe for farm animals by detoxifying the grain with anhydrous ammonia which reacts with aflatoxin molecules to destroy its toxicity have also not been adopted [76]. Such treatment, which must be done by trained personnel to avoid ammonia smell in the feed, can reduce aflatoxin by 95% [76]. No methods of analysis exist for detecting zero tolerance; therefore, tolerance levels should be based on a risk assessment approach rather than on analytical detection limits, the limits below which no detection is possible by analytical means [76]. This has not yet been achieved, and therefore a 'below detection limit' reported is just a product of instrument sensitivity and preparation losses but does not preclude trace level contamination and human exposure. So far more and more research have been concentrated on determining aflatoxin prevalence and levels in various foods, but research focus needs to shift towards effects and the use of biomarkers as well as epidemiological studies to understand the health impacts in Kenya.

Human exposure from milk has been a major issue of concern [113, 115, 116]. This originates from feeding cows with contaminated feeds or encouraging unhygienic conditions during milking, handling and storage of milk. Dairy production is widely practised in Kenya, and it provides a source of income to farmers, animal feed industry workers and all other stakeholders within the value chain [116]. Dairy farming systems in Kenya have changed over the years from direct use of pastures and hay only to commercial type of animal feeding where cowshed feeding is achieved with grain-based concentrates and silage [103, 105]. This practice was adopted due to increased productivity and high demand for the product. Studies have shown that aflatoxin contamination occurs in commercial feeds in Kenya and that exposure of cattle to mycotoxins generally occurs through consumption of contaminated feeds [103, 105, 109, 117]. AFM1 is usually excreted after 12 h in milk and urine when animal feed contaminated with AFB1/AFB2 is administered to the animals [22]. Aflatoxin is highly toxic to livestock, and feed contamination has been linked to increased mortality in farm animals. When cows consume aflatoxin-contaminated feed, they biotransform approximately 3–6% of AFB1 and AFB2 in their liver by hepatic microsomal mixed-function oxidase enzyme system into hydroxylated metabolites AFM1 and AFM2 [118] which are secreted into milk. AFB1, AFM1 and AFM2 aflatoxins have been detected in cow milk in Kenya [105]. Although AFM1 is 1000 times less toxic compared to AFB1, the AFM1 levels are regulated, and milk containing above 0.5 ppb level of AFM1 is considered unfit for human consumption [117]. Many countries have therefore regulated levels of AFB1 in animal feed, and the EU maximum limit has been set to 5 ppb; and it is recommended that animals should consume less than 40 μg/day of AFB1 in order not to exceed the allowed limit of AFM1.

7. Aflatoxin B1 regulation in Kenya

The World Health Organization, in collaboration with the Food and Agriculture Organization, is responsible for assessing the risks to humans caused by mycotoxins through contamination in food and for recommending adequate maximum levels in food and feed. Risk assessments of mycotoxins in food done by the Joint FAO/WHO Expert Committee on Food Additives are used by governments and by the Codex Alimentarius Commission (the intergovernmental standard-setting body for food) to establish maximum levels in food and provide other risk management advice to control or prevent contamination [11]. The outcome of such health risk assessments can either be a maximum tolerable intake (exposure) level or other guidance to indicate the level of health concern (such as the margin of exposure), including advice on risk management measures to prevent and control contamination and on analytical methods and monitoring and control activities [25]. These tolerable daily intakes are used by governments and international risk managers, such as the Codex Alimentarius Commission, to establish maximum levels for mycotoxins in food [11]. The maximum levels for mycotoxins in food are very low due to their severe toxicity. For example, the maximum levels for total aflatoxins set by the Codex in various nuts, grains, dried figs and milk are in the range of 0.5–10 μg/kg [24]. The WHO encourages national authorities to monitor and ensure that levels of mycotoxins in foodstuff on their market are as low as possible and comply with the both national and international maximum levels, conditions and legislation [25].

Different countries and authorities worldwide have rules and regulations governing aflatoxin B1 in foods which include the maximum permissible levels and recommended levels for certain foods. The Kenya Bureau of Standards (KEBS) has adopted the broad Codex standard limits of 5 ppb (for single metabolite) and 10 ppb for total aflatoxins in food but does not have lower limits for sensitive foods such as milk. The US Food and Drug Administration (FDA) had given an action level (maximum permissible) of total aflatoxin (B1) in combination with B2, G1 and G2 in foods as 20 μg/kg above in which the commodity is withdrawn from the markets [59], except milk which has a maximum level of 0.5 ppb. The Food Standards Agency has set a legal limit of total aflatoxins in foods as 10 μg/kg. Higher levels of 100–300 μg/kg are tolerable for some animal feeds. The EU has set maximum permitted levels for aflatoxin B1 in nuts, dried fruits, cereals and spices ranging from 2 to 12 μg/kg, while the maximum permitted level for aflatoxin B1 in infant foods is set at 0.1 μg/kg [119]. The maximum permitted levels for aflatoxin B1 in animal feeds set by the EU range from 5 to 50 μg/kg, and these levels are much lower than those set in the USA [120]. The Joint FAO/WHO Expert Committee on Food Additives has set the maximum permitted total aflatoxin level of AFB1 in combination with the other aflatoxins (B2, G1 and G2) at 15 μg/kg in raw peanuts and 10 μg/kg in processed peanut, while the tolerance level of aflatoxin B1 alone is 5 μg/kg for dairy cattle feed [121, 124, 125]. Results from previous studies have however shown that it is difficult if not impossible to eradicate AFB1 in cereals once produced [26]. For that matter, consumers are left vulnerable to exposure, yet burning of contaminated cereals, one of the most feasible ways of containing the menace, has caused problem of food insecurity in the past.

8. Conclusions

Aflatoxicosis cases are very common in Kenya, and the major cause is contaminated maize and maize flour. The total aflatoxin and AFB1 levels that have been obtained in maize grains and maize flour are indicating that commercial milling

and packaging of maize flour reduce the levels of aflatoxins considerably. However, a large population in the rural and urban areas which still rely on maize flour from open markets, through donation or by traditional posho milling, could be more exposed to aflatoxins as these sources increase and fail to reduce the levels, respectively. More research is needed to identify and determine aflatoxin levels in other produce such as beans, peanuts, groundnuts and their processed products. The current KEBS regulation and maximum allowable limits, in terms of total or single metabolite, are adequate for monitoring and controlling aflatoxicosis menace; however, for export produce and for long-term control of aflatoxicosis in the country, the maximum allowable limits need to be reviewed and lowered. With improvements in analytical techniques which are capable of giving lower detection limits, maximum allowable limits can be lowered to almost zero tolerance to reduce aflatoxicosis and hepatocarcinogenesis in human in Kenya. Although a lot of research in Kenya has gone into identification and determination of aflatoxin levels in various human foods and animal feeds and their detoxification mechanisms, it is still not possible to directly link AFB1 exposure to liver cancer as less epidemiological and biomarker studies have been done in Kenya to confirm such linkage.

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