Risk assessment and risk management of mycotoxins

Summary

Risk assessment is the process of quantifying the magnitude and exposure, or probability, of a harmful effect to individuals or populations from certain agents or activities. Here, we summarize the four steps of risk assessment: hazard identification, dose–response assessment, exposure assessment, and risk characterization. Risk assessments using these principles have been conducted on the major mycotoxins (aflatoxins, fumonisins, ochratoxin A, deoxynivalenol, and zearalenone) by various regulatory agencies for the purpose of setting food safety guidelines. We critically evaluate the impact of these risk assessment parameters on the estimated global burden of the associated diseases as well as the impact of regulatory measures on food supply and international trade. Apart from the well-established risk posed by aflatoxins, many uncertainties still exist about risk assessments for the other major mycotoxins, often reflecting a lack of epidemiological data. Differences exist in the risk management strategies and in the ways different governments impose regulations and technologies to reduce levels of mycotoxins in the food-chain. Regulatory measures have very little impact on remote rural and subsistence farming communities in developing countries, in contrast to developed countries, where regulations are strictly enforced to reduce and/or remove mycotoxin contamination. However, in the absence of the relevant technologies or the necessary infrastructure, we highlight simple intervention practices to reduce mycotoxin contamination in the field and/or prevent mycotoxin formation during storage.

1. Introduction

This chapter covers two key topics related to mycotoxins in human food: risk assessment and risk management. Managing risks of food contaminants such as mycotoxins is of global importance. Indeed, two international policy-making bodies – the United Nations Environment Programme (UNEP) and the World Health Organization (WHO) International Programme on Chemical Safety (IPCS) – have declared that humans have a right to food free from mycotoxins that could cause significant health risk. In highly populated parts of the world, mycotoxins in staple crops remain the most significant foodborne risk for human health, animal health, and market access (FAO/WHO/UNEP, 1999).

Risk assessment and risk management of mycotoxins deserve much more global attention and action than

they have been given. Wild and Gong (2010) identified several reasons for current inaction in addressing mycotoxin risks in developing countries. First, knowledge about mycotoxins and the full range of their risks to health is incomplete. Second, risks are poorly communicated to policy-makers in regions where mycotoxins are most prevalent. Third, the perceived value of interventions to reduce mycotoxin risk is low compared with those of other medical interventions, such as vaccination programmes, malaria control, and improved sanitation. Fourth, intervention to control mycotoxins is required at multiple time points both before and after harvest. Fifth, regulation of mycotoxins has minimal effects on food quality for subsistence farmers. Finally, mycotoxin contamination is a problem that encompasses agriculture, health, and economics, fields that traditionally have not interacted at a research or policy-making level.

This chapter covers both the theoretical and quantitative aspects of risk assessment, to provide a background in how human health risks are assessed both experimentally and for decision-making purposes. Section 2 outlines the four steps in the risk assessment process and describes how risk assessments have been conducted for five mycotoxins: aflatoxins, fumonisins, ochratoxin A, deoxynivalenol, and zearalenone. Section 3, on risk management, is more practical and focuses specifically on how to manage mycotoxin risks in foods. Finally, Section 4 briefly describes the importance of risk communication and public education about mycotoxins in countries at risk.

2. Risk assessment

Risk assessment is the process of estimating the magnitude and probability of a harmful effect to individuals or populations from certain agents or activities. Four steps are involved in estimation of risk: hazard identification, dose–response assessment, exposure assessment, and risk characterization (NRC, 2008).

2.1 Hazard identification

Hazard identification is the process of determining whether exposure to an agent can increase the incidence of a particular adverse health effect. A variety of different studies can help to determine whether an agent causes a specific health effect; these include studies of adverse effects in humans, animals, and in vitro systems, and mechanistic studies.

Epidemiological studies on the relationship between exposure to a mycotoxin and particular harmful effects obviously provide the best evidence for identifying a human health hazard. Studies that provide a quantitative estimate of association between a hazard and a disease include cohort and case–control studies. Other types of human studies, which may or may not provide a quantitative estimate of association, include studies that are descriptive (case reports), ecological (geographical comparison), and cross-sectional (observing disease prevalence across different populations at a single time point).

In a cohort study, an investigator selects a group of initially healthy individuals (a cohort) exposed to any number of potentially hazardous agents, and follows individuals in this group over time to determine the incidence of a particular disease that may be associated with particular exposures. Then, the incidence of the disease in exposed individuals is compared with the incidence in unexposed individuals, or the incidence in a highly exposed group may be compared with the incidence in a group exposed to lower levels. For example, it may emerge that over time, the incidence rate of a particular cancer is higher in a group of individuals exposed to higher levels of a particular mycotoxin than the rate in a group of individuals exposed to lower levels of that mycotoxin, or not exposed at all. A cohort study can be used to estimate the relative risk (RR) of a particular disease in exposed versus unexposed populations:

RR = (Incidence of disease in the exposed population)/(Incidence of disease in the unexposed population).

In the exposed group, if the number of people who develop the disease is a and the number of people who do not develop the disease is b, then the incidence of disease in the exposed population is $a/(a + b)$. Likewise, in the unexposed group, if the number of people who develop the disease is c and the number of people who do not develop the disease is d, then the incidence of disease in the unexposed population is $c/(c + d)$.

In a case–control study, an investigator identifies a group of individuals with a particular disease (cases) and a comparable group of individuals without that disease (controls), and determines what proportion of the cases were exposed and what proportion were not (Gordis, 2009). The assumption is that if the proportion of individuals exposed to an agent is different between cases and controls, then exposure to the agent may be associated with an increased or decreased occurrence of the disease. A case–control study can be used to estimate an odds ratio (OR) to compare the odds that cases were exposed to the agent with the odds that controls were exposed:

OR = (Odds that a case is exposed)/(Odds that a control is exposed).

Using the same variables described above to estimate RR, the

odds that a case is exposed is $a/(a + c)$ and the odds that a control is exposed is $b/(b + d)$.

More recently, attention has focused on whether it is possible to take advantage of the benefits of both of these study types by combining elements of both cohort and case–control approaches into a single study. For example, a case–control study can be initiated within a cohort study, i.e. the individuals within the cohort who develop a particular disease during the study period can be considered cases and those who do not develop the disease can be considered controls. In nested case–control studies, the controls are a sample of individuals who are at risk for the disease at the time each case of the disease develops (Gordis, 2009).

2.1.1 Human studies

Human studies for the purpose of risk assessment can be problematic, for several reasons. First, epidemiological studies exist for only a few hazardous agents. Second, circumstances of human exposure levels and duration are difficult to measure precisely, and there is often a time interval between when exposure occurs and disease symptoms appear. Third, even well-planned studies cannot always show a clear causative association between an agent and a disease. Many potential confounders exist that can contribute to the disease etiology, such as exposure to multiple environmental disease agents, varied nutritional status, and genetic factors that modulate disease susceptibility. Finally, human studies may be susceptible to bias or systematic errors in the design, conduct, or analysis of the studies that result in mistaken estimates of the effect of an exposure on disease risk (Gordis, 2009). For example, there may be selection bias in how individuals are chosen for or respond to a study, or information biases such as those associated with interviewing, surveillance, recall, or reporting.

2.1.2 Animal studies

Animal studies (in vivo) are often used in place of human studies for hazard identification, for reasons described above. The major concern with animal studies is that extrapolation from results in another species to results in humans is imperfect. However, animal experiments have several advantages: dose levels of the hazardous agent may be varied, other conditions may be kept constant across all groups to reduce confounders, and an animal group can specifically be kept unexposed as controls. Then, the incidence of a health effect can be compared across these groups.

2.1.3 Cell and tissue culture studies

Cell and tissue culture assays, unlike human and animal studies, are in vitro assays, and these are increasingly important experiments for identifying hazards. Isolated cells or tissues (or microorganisms) are prepared and maintained in culture by methods that preserve some in vivo properties, and, after exposure to a hazardous agent, they can be tested for point mutations, chromosomal aberrations, DNA repair or damage, gene expression, cell transformation, and metabolic and other physiological effects. These tests may give information on potential mechanisms of the biological endpoint (e.g. an adverse health effect).

Structure–activity relationship (SAR) models allow the toxicological activity of an unknown chemical or agent to be predicted on the basis of its chemical and/or structural properties. The relationships are computationally derived from information about agents of known toxicity. These predictive equations are most useful in setting priorities for further research, thereby reducing the number of animal experiments required. Requirements for SAR modelling thus include a database of chemicals of known toxicity or carcinogenicity, information about their chemical and spatial structure, and information about their physicochemical properties.

2.2 Dose–response assessment

The second step in risk assessment, dose–response assessment, determines the relationship between the dose of a toxic agent and the occurrence of health effects. This relationship is often graphically represented in a dose–response curve, which shows the proportion of a given population that experiences an adverse health effect (on the vertical axis) at different doses of a toxic agent (on the horizontal axis). The shape of the dose–response curve is a critical component in policy-making to control human health risks.

Usually, dose–response data are gathered from animal and/or human studies. Animal studies are especially useful for dose–response assessment because the exact doses of a particular toxin can be carefully controlled. Different groups of animals are exposed at each dose level through food, water, air, or dermal contact, and one group of animals is not exposed to the toxin at all (control group). The prevalence of a health effect, or the lack thereof, is measured in each animal group at each dose level, and these results are plotted in a curve.

Dose–response assessment addresses toxic (non-carcinogenic) and carcinogenic effects separately and differently, even if an agent can cause both types of effects (e.g. aflatoxin). These effects are briefly described here.

2.2.1 Toxic (non-carcinogenic) effects

For toxic but non-carcinogenic effects caused by an agent, it is assumed that protective mechanisms must be overcome before the adverse health effect can occur. A threshold dose of the toxin may exist, i.e. humans and animals can be exposed to the toxin at doses below this particular threshold without experiencing the health effect in question. The threshold dose is referred to as the no-observed-effect level (NOEL), sometimes called the no-observed-adverse-effect level (NOAEL). In animal studies, the NOEL is the highest dose of a toxin at which no significant increases in the frequency or severity of adverse effects are observed when an exposed group is compared with an unexposed group. Sometimes a lowest-observed-effect level (LOEL), the lowest dose in the experiment at which an adverse effect can be observed, is used in place of or in addition to the NOEL.

The NOEL is one metric used to evaluate the toxicity of an agent. Another is the median lethal dose (LD_{50}), the dose of a toxin that, when administered to a group of animals over a specified period of time, is lethal to 50% of the animals. LD_{50} values are used to rank toxicity across multiple agents. Other metrics include the 10% lethal dose (LD_{10}), the dose of a toxin lethal to 10% of test animals, and the median effective dose (ED_{50}), the dose that causes a particular health effect – not necessarily death – in 50% of test animals.

More recently, benchmark dose modelling – which uses the entire dose–response curve from an animal study – has been used to provide a different point of departure from the NOEL in calculating a tolerable daily intake (TDI). Benchmark dose modelling involves finding a model that best fits the overall shape of the dose–response curve in an animal study and then, from that model, finding the dose that corresponds to a proportion (usually 10% or 5%) of response in the test animals. This particular dose is called the benchmark dose. Then, the lower bound of the confidence interval around that dose (from uncertainties in the animal study itself) is calculated. This benchmark dose lower confidence limit ($BMDL_{10}$ or $BMDL_{05}$) is used as the point of departure for extrapolating to a TDI for humans.

To extrapolate animal toxicity data to humans, a provisional maximum tolerable daily intake (PMTDI), also called a reference dose (RfD) in some cases, is calculated. The PMTDI is the dose below which humans exposed to the agent in question are not expected to experience adverse health effects. The PMTDI is derived from the NOEL or BMDL by applying uncertainty factors, which can account for interspecies variability (extrapolating from animals to humans), intraspecies variability (to protect sensitive individuals), use of a LOEL instead of a NOEL, the chronicity of the study, and other terms reflecting the professional assessment of additional uncertainties in the data. The PMTDI is calculated by dividing the NOEL or BMDL by the product of the uncertainty factors:

PMTDI = NOEL/(product of uncertainty factors), or

PMTDI = BMDL/(product of uncertainty factors).

Often, the uncertainty factors for interspecies and intraspecies variability are each given the value of 10. Therefore, the human PMTDI for a non-carcinogen is usually 0.01 times the NOEL or BMDL found in animal studies. It is worth noting that for policy-making purposes, this type of extrapolation from an animal study to a human PMTDI has often caused controversy because of the arbitrary nature of choosing uncertainty factors. Practically speaking, the safety factor assumes that humans are 10 times as sensitive as the most sensitive animal species tested for a particular toxin and that the most sensitive human is 10 times as sensitive as the least sensitive human.

2.2.2 Carcinogenic effects

In contrast to most toxic effects, carcinogenesis may be regarded as a process in which the presumption of no threshold may be appropriate if the chemical is (directly acting) or its metabolites are (indirectly acting) reactive with genomic DNA (Klaunig and Kamendulis, 2008). For such carcinogens, theoretically even a single molecular event could evoke changes in genomic DNA leading to mutations, selective cellular proliferation, and cancer. Hence, it is assumed that there is no safe dose above zero. For example, it is assumed that there is no threshold of exposure to aflatoxin B_1 below which cancer would never occur, because aflatoxin B_1 has a reactive metabolite that interacts directly with DNA. Experiments in rats exposed to aflatoxin B_1 in their drinking-water showed that the level of DNA adducts in the liver was linear over 6 orders of magnitude after both single and chronic dosing; dose levels reached those seen in exposed human populations (Buss *et al.*, 1990). Additional mechanisms of carcinogenicity may exist that do not involve genotoxicity, which could be relevant for other mycotoxins.

For policy-making purposes, carcinogens are evaluated in two parts. First, a weight of evidence (WOE) is designated, and second, a slope factor, or cancer potency factor, is calculated on the basis of the dose–response

curve. WOE is a term that refers to the strength of the evidence that a particular agent causes cancer in humans. Two organizations that provide WOE evaluations for carcinogens are the International Agency for Research on Cancer (IARC) and the United States National Toxicology Program (NTP). IARC classifies agents as to carcinogenicity to humans by considering the WOE (Table 7.1).

For example, IARC has classified naturally occurring mixtures of aflatoxins as Group 1, carcinogenic to humans, and fumonisin B_1 and ochratoxin A as Group 2B, possibly carcinogenic to humans.

For carcinogenic agents that are reactive with DNA, a slope factor, or cancer potency factor, is calculated by estimating the slope of the linearized dose–response curve. Sometimes, for policy-making purposes, the slope factor is actually the upper 95% confidence limit of the dose–response slope. In practical terms, the slope factor or cancer potency factor estimates the increase in probability of developing the particular cancer per unit dose of the agent over a human lifetime. Thus, the steeper the dose–response curve for a carcinogenic agent, the more potent it is in causing cancer. For carcinogens that do not directly damage DNA, the NOEL can be used for setting a PMTDI. For example, the long-term nephrotoxicity of fumonisin B_1 is a prerequisite for the renal carcinogenicity of fumonisin B_1 (Dragan *et al.*, 2001). In this case, the renal carcinogenicity is subsumed by the dose–response relationship for the nephrotoxicity, which is clearly a threshold event (Bolger *et al.*, 2001).

2.3 Exposure assessment

The third step in risk assessment, exposure assessment, is the process of estimating the intensity, frequency, and duration of human or animal exposures to an agent in the environment. Exposure assessment

Table 7.1. IARC classification of agents as to carcinogenicity to humans based on the weight of evidence

Category	Significance
Group 1	Carcinogenic to humans
Group 2A	Probably carcinogenic to humans
Group 2B	Possibly carcinogenic to humans
Group 3	Not classifiable as to its carcinogenicity to humans
Group 4	Probably not carcinogenic to humans

is also a critical component of all epidemiological studies and is often used to identify control options or technologies to reduce risk.

2.3.1 Calculating exposure

Three steps are involved in calculating exposure: (i) characterizing the exposure setting, (ii) identifying exposure pathways, and (iii) quantifying exposure.

To characterize an exposure setting, both the physical setting and the exposed populations must be understood. The physical setting includes factors such as climate, geographical setting, vegetation, soil type, and location of water. Potentially exposed populations include both humans and animals: those living nearest the risky agent; those with diets, water, or air supply containing the agent; those who come to an area near the risky agent for work or play; and any other demographic groups (e.g. based on age or sex) that would be disproportionately exposed to the agent.

Characterizing an exposure pathway involves identifying sources and points of contact, media that transport the agent to the population, ways in which the agent may react or change in transport media, and other physical and chemical properties of the agent that explain its fate as it moves along its pathway to a target population.

Finally, to quantify exposure, it is common to estimate an average

daily dose (ADD) or intake. This term is usually expressed as the mass of substance in contact with the body per unit body weight (bw) per unit time, such as in mg/kg bw/day, for ingestion exposures, or as the mass of substance per cubic meter of ambient air, such as in mg/m^3, for inhalation exposures. If the agent being studied is a carcinogen, a lifetime average daily dose (LADD) is calculated, with an averaging time equal to the expected lifetime of the individual (e.g. the United States Environmental Protection Agency assumes an averaging time for cancer risk assessment of 70 years, although the average lifespan in the USA is now longer than this).

To calculate ADD or LADD, the exposure quantity E must first be estimated. It is the concentration of an agent as a function of time t, over an exposure duration. The total exposure quantity during that given time is expressed as the integral (sum) of concentrations C over the exposure duration:

$$E = \int C(t) \, dt.$$

However, the integrated concentration can be difficult to obtain, so instead one can estimate the arithmetic average of the concentration C_{ave} over the exposure duration ED to estimate the total exposure:

$$E = C_{ave} * ED.$$

The intake rate *IR* is the amount of the agent passing through the initial intake barrier (mouth, nose, skin) into the body over a period of time. This can be measured in mg/day or L/day, for example. Then, the ADD is calculated as:

$$ADD = (E * IR)/(BW * AT),$$

where *BW* is body weight and *AT* is the averaging time in days. LADD is calculated similarly; in this case, however, the exposure duration (used to calculate *E*) is the number of years that an individual is exposed to a carcinogen, and the averaging time *AT* is the expected lifetime in years.

2.3.2 Estimating human exposure to mycotoxins

Many difficulties have been encountered in estimating mycotoxin exposure in human diets. Until recently, human exposure to mycotoxins was measured almost exclusively in one of two ways: by questionnaires or food diaries relying on recall of what and how much had been eaten; or by food samples collected from populations, which ideally were representative of true exposures. Both of these ways pose potential problems. Dietary recall is often inaccurate. It can be difficult in many cultures worldwide to take food samples without disturbing social contexts, and measurement may lead to abnormal eating behaviour during recording. In addition, snack foods may be a significant source of exposure to mycotoxins, but these may not be recalled and may not be measured (Hall and Wild, 1994).

In recent years, however, biomarkers to assess mycotoxin exposure, internal dose, and biologically effective dose have been developed and are increasingly being used to estimate human exposure (Groopman *et al.*, 2008). Biomarkers

can also be used to assess the effectiveness of interventions to reduce mycotoxin exposure. Measurement of biomarkers related to mycotoxin exposure typically requires samples of either urine or serum. As described in Chapter 6, the measurement of validated biomarkers for aflatoxin exposure in human populations has greatly assisted epidemiological studies. Validated biomarkers for fumonisins and deoxynivalenol have also been developed. However, a past occurrence of measurement of an unvalidated biomarker for ochratoxin A resulted in inaccurate representation of actual dietary intake (Gilbert *et al.*, 2001). Moreover, collection of samples for measuring biomarkers in human populations may also pose cultural challenges.

Without accurate exposure data, quantitative risk assessments can be limited, because exposure is a major component of the calculations. Hence, it is important to ensure that measurements are carried out in the most accurate and reliable way possible.

2.4 Risk characterization

Risk characterization, the final step in risk assessment, combines the information on exposure with that on toxicity and dose–response assessment to determine whether an individual or a population is experiencing a significant risk of illness or disease based on exposure to a hazardous agent. It translates the available data to describe this significance to a broad audience.

Risk characterization, like dose–response assessment and exposure assessment, is conducted differently for non-carcinogens and carcinogens. For toxic effects, the individual or population ADD of an agent is compared with the PMTDI of the agent. For carcinogenic effects, the individual or population LADD is compared with

the dose–response information from the specific agent. Discussion of major assumptions, scientific judgements, and estimates of uncertainties should also be part of the final risk characterization.

2.4.1 Risk characterization of non-carcinogenic toxins

Simplistically, determining whether an individual or a population may suffer a health risk from a hazard relies on knowing whether their average daily exposure is greater than the daily dose of the hazard that may cause adverse effects. To recapitulate: the PMTDI is estimated from dose–response assessments that determine a NOEL or BMDL of a particular hazardous agent. The PMTDI is obtained by dividing the NOEL or BMDL by uncertainty factors that take into account extrapolation from laboratory animals to humans and variation among humans. Thus, the PMTDI is an estimate of a daily exposure level for humans, including sensitive individuals, that is unlikely to cause adverse health effects. The ADD can be calculated based on human exposure to the hazardous agent.

If ADD > PMTDI, then the potential for health risk exists. One way to express this is the hazard quotient (HQ) used by the United States Environmental Protection Agency. HQ is calculated as the ratio of the ADD of a particular agent to the PMTDI of that same agent:

$$HQ = ADD/PMTDI.$$

If HQ > 1, then an individual or a population may suffer a health risk due to their levels of exposure to the hazardous agent. If HQ < 1, then the individual or population is unlikely to suffer a health risk from current exposure levels to the agent.

2.4.2 Risk characterization of carcinogens

Rather than using a threshold, such as HQ in the evaluation of non-carcinogens, carcinogenic risk is estimated for an individual or a population over an expected lifetime. Policy-makers may then determine whether this expected lifetime risk is acceptable or whether the carcinogen should be regulated. The WOE, based on available studies as described above, also factors into policy decision-making about the carcinogenic agent in question.

To reiterate: the slope factor of a carcinogen is derived by taking the slope of the linearized dose–response curve. LADD is estimated based on an exposure of an individual to the carcinogenic agent. Then,

$$Risk = LADD * slope\ factor,$$

where *Risk* is a unitless probability of an individual developing cancer over a lifetime (for example, 3 in a million, or 3×10^{-6}) from being exposed to the carcinogenic agent.

2.5 Risk assessment of mycotoxins

A detailed discussion of the human health risks associated with aflatoxins, fumonisins, ochratoxin A, deoxynivalenol, and zearalenone is given in Chapter 6. In this section, we go into more detail about risk assessment of each of these mycotoxins for the purposes of policy-making.

2.5.1 Aflatoxins

The evidence for aflatoxins causing liver cancer (hepatocellular carcinoma [HCC]) in humans has been established from decades of epidemiological research. These studies have elucidated dose–response relationships from which

quantitative cancer risk assessments can be conducted (WHO, 1998). Based on the WOE of the effects of aflatoxins in human, animal, and in vitro studies, IARC has classified naturally occurring mixtures of aflatoxins as Group 1, carcinogenic to humans (IARC, 2002). However, the carcinogenicity, or cancer potency, of aflatoxins differs in humans with and without chronic hepatitis B virus (HBV) infection. The risk of HCC attributable to aflatoxins is up to 30-fold higher in populations chronically infected with HBV than in uninfected populations (Groopman *et al.*, 2008).

In 1998, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) undertook an aflatoxin and HCC risk assessment to estimate the impact on population HCC incidence of moving from a hypothetical total aflatoxin standard of 20 µg/kg to a stricter standard of 10 µg/kg (WHO, 1998; Henry *et al.*, 1999). Assuming that all food that contained aflatoxin levels higher than the standard would be discarded and that enough maize and nuts would remain to preserve consumption patterns, JECFA determined that HCC incidence would decrease by about 300 cases per billion people per year if the stricter aflatoxin standard were applied in countries with an HBV prevalence of 25%. However, in countries where the HBV prevalence was 1%, using the stricter aflatoxin standard would decrease HCC incidence by only 2 cases per billion people per year. This assessment associated HCC risk with particular doses of aflatoxin. However, these doses do not correspond to actual doses in food in different parts of the world, and two hypothetical values for HBV prevalence were assumed: 1% and 25% (Liu and Wu, 2010).

Liu and Wu (2010) estimated the global burden of HCC induced by aflatoxin by using the quantitative cancer risk assessment described above and collecting national data on foodborne aflatoxin levels,

consumption levels of maize and groundnuts, and HBV prevalence. The cancer potencies of aflatoxin for HBV-positive and HBV-negative individuals were considered, together with uncertainties in all variables. Liu and Wu estimated that of the 550 000 to 600 000 new HCC cases worldwide per year, about 25 200 to 155 000 may be attributable to aflatoxin exposure. In other words, aflatoxin may play a causative role in 4.6–28.2% of all global HCC cases. Most cases occur in sub-Saharan Africa, South-East Asia, and China, where populations suffer from both high HBV prevalence and largely uncontrolled exposure to aflatoxin in food.

2.5.2 Fumonisins

Compared with the risk assessment of aflatoxins, risk assessments of fumonisins and ochratoxin A for risk management are more complex from the human health standpoint because of the lack of convincing evidence linking either mycotoxin to human disease (WHO, 2001b) and, in the case of ochratoxin A, the controversy over its mechanism of action (WHO, 2001b).

Gelderblom *et al.* (2001) estimated the NOAEL for HCC induction by fumonisin B_1 in male rats at 0.8 mg/kg bw/day, which translated to a TDI of 0.8 µg/kg bw/day when divided by a safety factor of 1000. The justification for the large safety factor is that although fumonisin does not directly damage DNA, it induces cancer in rats and mice (IARC, 2002) and is a cancer promoter (Carlson *et al.*, 2001; Gelderblom *et al.*, 2002). Adopting such a standard, however, could seriously affect the food supply and trade between producers and consumers (Steyn *et al.*, 2008).

JECFA set and then retained a PMTDI for fumonisins of 2 µg/kg bw/day, based on one evaluation of a NOAEL for nephrotoxicity in male

rats and a safety factor of 100 (WHO, 2001b), and then more recently based on a benchmark dose ($BMDL_{10}$) that causes megalocytic hepatocytes in male mice and a safety factor of 100 (WHO, 2011). The safety factor of 100 was deemed appropriate because the mechanism of toxicity and carcinogenicity did not involve direct damage to DNA. IARC has classified fumonisin B_1 as Group 2B, possibly carcinogenic to humans (IARC, 2002).

2.5.3 Ochratoxin A

The mechanism of carcinogenicity of ochratoxin A (OTA) remains unclear. Six hypotheses about this mechanism were listed by JECFA at its most recent evaluation (WHO, 2008). Positive results for genotoxicity were usually obtained only at high OTA exposure levels and were usually indicative of oxidative damage. JECFA concluded that a direct genotoxic mode of action remains unconfirmed and that several possible modes of non-genotoxic action could be involved in the formation of renal tumours induced by OTA. Non-carcinogenic effects may occur at lower levels that those inducing tumour formation, however. On the basis of a study on damage to renal function in pigs (Krogh *et al.*, 1974) and a safety factor of 500, JECFA established a provisional tolerable weekly intake (PTWI) for OTA of 112 ng/kg bw/week (WHO, 1991). This finding was confirmed by JECFA in 1995, when the PTWI was rounded off to 100 ng/kg bw/week (WHO, 1995). For the most recent evaluation, JECFA modelled carcinogenicity data on OTA from rat bioassays performed by the United States National Toxicology Program (NTP, 1989). Six different models were used on data on carcinoma in the male rat kidney, the most sensitive sex, species, and target organ. Risk assessment indicated that acute toxicity occurred at lower levels

than did long-term effects such as carcinogenicity, so the previous PTWI, set on the basis of nephrotoxicity before carcinogenicity was definitely established, was retained (WHO, 2008). IARC has classified OTA as Group 2B, possibly carcinogenic to humans (IARC, 1993).

The current average dietary exposure levels to OTA have been determined by JECFA to be 8–17 ng/kg bw/week (WHO, 2008). These levels are well below the PTWI; however, JECFA had previously determined (WHO, 2001b) that the 95th percentile for OTA consumption was about 84–92 ng/kg bw/week, approaching the PTWI. A recent reappraisal of the risk associated with OTA resulted in a reduced PTWI of 21 ng/kg bw/week, one fifth of the JECFA estimate, based on applying an even larger uncertainty factor to the study of Krogh *et al.* (1974) evaluated by JECFA (Kuiper-Goodman *et al.*, 2010).

Dietary exposure is based primarily on data from Europe, where processed cereal foods often show high levels of contamination with OTA (Steyn *et al.*, 2008; WHO, 2008). Contamination of cereals in Europe is due to *Penicillium verrucosum*, which is a fungus of cool climates and does not occur in the tropics. Although sometimes contaminated by *Aspergillus* species capable of producing OTA, cereals in tropical countries are not usually a major source. Coffee and cocoa are potential sources of OTA exposure in tropical countries (WHO, 2008).

2.5.4 Deoxynivalenol

The toxicity of deoxynivalenol (DON) has been reviewed by WHO and IARC, and risk assessments including toxicological reviews of DON have been published for Canada, the Nordic Council, the Netherlands, and the European Union (WHO,

2001). IARC has categorized DON as Group 3, not classifiable as to its carcinogenicity to humans (IARC, 1993). A 2-year study of mice exposed to DON suggested no carcinogenic hazard. Although the weight of the mice exposed to DON was lower than that of the controls, the difference was not considered biologically significant. JECFA established a PTMDI for DON of 1 µg/kg bw/day, based on the NOEL (for decreased body weight at day 500) in this mouse study of 100 µg/kg bw/day and a safety factor of 100 (WHO, 2001a).

2.5.5 Zearalenone

The toxicity of zearalenone (ZEA) was evaluated by JECFA in 2000. Reproductive and developmental effects, as well as estrogenic effects, were found in a variety of animal species, including rats, mink, and pigs (WHO, 2000). There is, however, little evidence for acute toxicity or carcinogenicity. In humans, ZEA was suspected to have caused premature thelarche in girls, but the evidence was inconclusive and other causative or contributing agents could not be ruled out (WHO, 2000). IARC has categorized ZEA as Group 3, not classifiable as to its carcinogenicity to humans (IARC, 1993). Using a safety factor of 80, JECFA set a PMTDI for ZEA of 0.5 µg/kg bw/day, based on the NOEL (for reversible increase in length of estrous cycle) of 40 µg/kg bw/day in a 15-day study in pigs (WHO, 2000).

3. Risk management

Risk assessments have confirmed health risks to human populations worldwide from several mycotoxins in food, including aflatoxins, fumonisins, ochratoxin A, and deoxynivalenol. Although several potential interventions exist by which to manage mycotoxin risks in food,

control is very difficult in practice. At this time, no single strategy enables risk from mycotoxins to be eliminated in any country.

Mycotoxin risks in food can be managed either by governmental regulations or by agricultural and public health interventions. Governments can impose food safety standards that specify a maximum tolerable limit of a particular mycotoxin in human food. At the same time, agricultural and public health interventions can be adopted to reduce mycotoxin levels in food or reduce the bioavailability of mycotoxins.

Mycotoxin standards have reduced foodborne mycotoxin risk in developed countries because enforcement is strong and because technologies and methods exist to successfully reduce or remove mycotoxin contamination. Commodities with moderately excessive levels of mycotoxins are removed from the food stream and used as feeds for animals that tolerate higher levels of mycotoxins, such as beef cattle, or as biofuels or fertilizers.

In low-income countries (LICs), the situation is quite different. In many parts of the world, regulatory standards for mycotoxins in food have little or no impact on actually reducing mycotoxin risk, for several reasons.

First, many rural farmers engage in subsistence farming, in which case food grown on farms is directly consumed by the families without ever undergoing a formal inspection process for mycotoxins. Second, even if regulatory standards exist for certain mycotoxins, there is often little to no enforcement of these standards in certain parts of the world. Third, if the regulatory standards are imposed by importing countries, farmers in LICs may export their best quality foods and keep the poorer quality for domestic use, inadvertently raising health risks related to mycotoxins in populations already vulnerable to disease because of poverty (Wu, 2004).

The risk of mycotoxin contamination of commodities and foods is greatest in LICs, where agricultural systems are often poorly equipped to handle food safety risks. Suboptimal field practices and poor storage conditions make the crops vulnerable to fungal infection and subsequent mycotoxin accumulation. The problem is exacerbated by the fact that maize and groundnuts, two of the food crops that are most susceptible to aflatoxin contamination, are staples in the diets of many people worldwide, and thus aflatoxin exposure is higher where dietary variety is difficult to achieve (Shephard, 2008). In good seasons, subsistence farmers and local food traders may be able to avoid eating obviously mouldy maize and groundnuts, but in drought seasons, or in situations of food insecurity, often people have no choice but to eat mouldy food or starve (Wu and Khlangwiset, 2010a). Indeed, 125 people died due to acute aflatoxicosis in rural Kenya in 2004 when food insecurity, caused by a variety of climatic and social factors, led to widespread consumption of maize contaminated with high levels of aflatoxins (Lewis *et al*., 2005).

One additional limitation of the setting of mycotoxin standards is that such standards are usually in the form of an allowable mycotoxin concentration in a particular food commodity, such as maize. Such a standard does not take account of the fact that some populations consume much more of the food commodity than other populations in the world; hence, those populations could be much more exposed even if regulatory standards were enforced.

For example, regulations on allowable fumonisin levels in maize may sufficiently protect populations that do not typically consume large amounts of maize, but the allowable levels may be too high in many parts of sub-Saharan Africa, where maize is a

dietary staple. There, populations may be exposed to potentially dangerous amounts of fumonisin from maize even if that maize meets regulatory standards. Table 7.2 highlights how people who have a very high maize intake can ingest dangerous levels of fumonisin even if the maize itself is considered relatively clean by regulatory standards, whereas people who consume very small amounts of maize could ingest a much more contaminated commodity without having significant fumonisin exposure (Gelderblom *et al*., 2008).

Hence, regulatory standards for mycotoxins in food sometimes have no impact, or even potentially adverse impacts, on human health in LICs (Wu, 2004; Shephard, 2008; Williams, 2008). Instead, the focus for risk management should be on <u>technologies</u> and public health interventions to reduce mycotoxin risk, <u>infrastructures</u> to support these technologies, and <u>public education</u> (Fig. 7.1).

Mycotoxins can be managed at various points along the food production chain from the field to the plate. In pre-harvest, or field, conditions, using good agricultural practices, such as choosing appropriate cultivars for the geographical region, can reduce the risk of fungal infection and subsequent mycotoxin accumulation. Post-harvest interventions involve careful sorting, cleaning, drying, storage, transportation, and processing to reduce the risk of further mycotoxin accumulation, or to lower mycotoxin levels directly.

Much of the work that has been done on dietary methods to reduce the bioavailability of mycotoxins has pertained specifically to aflatoxin. However, one simple dietary intervention that applies to all mycotoxins is, where feasible, to consume less of the foodstuffs that contain the mycotoxins. In the case of aflatoxin, that would mean consuming less maize and groundnuts if

possible, in favour of other food crops that have significantly lower aflatoxin contamination, such as rice, sorghum, or pearl millet. An example is the recent economic growth in China that has led to reduced maize consumption, and hence reduced aflatoxin exposure (Wild and Gong, 2010). Where it is not easy to make such a dietary shift (e.g. where maize and groundnuts have traditionally been staples), other dietary inter-ventions may prove helpful. These interventions are described in greater detail in Chapter 9.

One public health intervention does not result in lower mycotoxin levels but can reduce the adverse effects associated with HCC induced by aflatoxin: vaccination against HBV. A regular practice now in developed countries, HBV vaccination in children still requires wider implementation. Vaccinating children against HBV over the past three decades has resulted in significantly decreased HBV infection rates in several countries and regions, including Europe (Williams *et al.*, 1996, Bonanni *et al.*, 2003); Taiwan, China (Chen *et al.*, 1996); and Thailand (Jutavijittum *et al.*, 2005). This vaccine may have significant impacts on HCC incidence, particularly in Africa and Asia, where the current prevalence of chronic HBV infection is relatively high. To date, a reduction in HCC incidence resulting from HBV vaccination has been demonstrated in children and adults in Taiwan, China, after vaccination was introduced in 1984 (Chang *et al.*, 2009). By lowering the prevalence of chronic HBV infection, this vaccination should prevent the synergistic interaction between HBV and aflatoxin in inducing HCC (Khlangwiset and Wu, 2010; Liu and Wu, 2010).
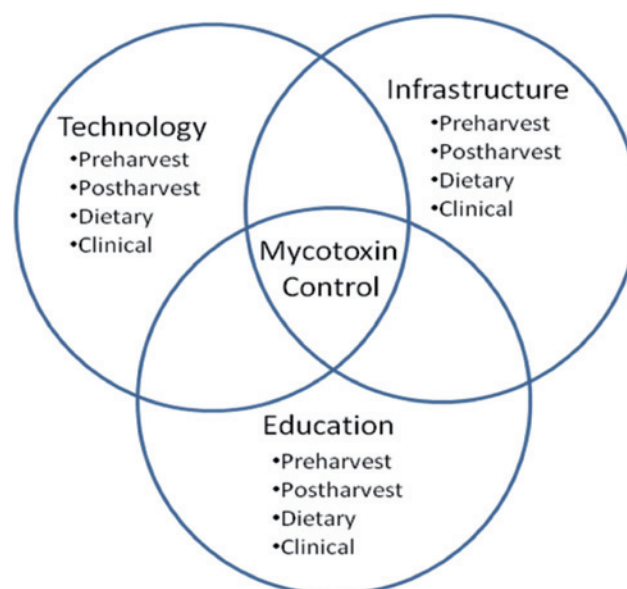
Table 7.2. Probable daily intake of fumonisins (µg/kg bw/day) as a function of maize intake and fumonisin contamination levels in food

Fumonisin level (mg/kg)	Maize intake (g/person [60 kg]/day)[a]						
	10	50	100	150	200	400	500
0.2	0	0.2	0.3	0.5	0.7	1.4	1.7
0.5	0.1	0.4	0.8	1.3	1.7	3.4	4.2
1	0.2	0.8	1.7	2.5	3.3	6.6	8.3
2	0.3	1.7	3.3	5.0	6.7	13.4	16.7
3	0.5	2.5	5.0	7.5	10.0	20.0	25.0
4	0.7	3.3	6.7	10.0	13.3	26.6	33.3

[a] The shaded values are those intakes closest to the Joint FAO/WHO Expert Committee on Food Additives (JECFA) provisional maximum tolerable daily intake (PMTDI) of 2 µg/kg bw/day for fumonisin B_1 (FB_1), FB_2, and FB_3 alone or in combination.
Adapted, by permission of the publisher, from Gelderblom *et al.* (2008).

Fig. 7.1. Mycotoxin control in developing countries. In the absence of enforcement of mycotoxin standards, technology, appropriate infrastructure, and public education are required to implement strategies that reduce mycotoxin risk.

4. Mycotoxin risk and public education

There is no question that mycotoxins in food pose a significant public health risk in many parts of the world. Risk assessments conducted using human, animal, and in vitro studies and exposure assessments have confirmed the reality of this global health problem. HCC is a leading cause of cancer deaths worldwide (Ferlay et al., 2008), and an HCC risk assessment reveals that aflatoxin may play a causative role in more than one quarter of HCC cases (Liu and Wu, 2010). Epidemiological studies have also linked aflatoxin exposure with stunted growth in children, acute aflatoxicosis, and liver cirrhosis and have suggested fumonisin exposure as a possible risk factor for oesophageal cancer and neural tube defects (Wild and Gong, 2010).

In developed countries, mycotoxin exposure in the diet is controlled by good agricultural practices, good storage and processing, and control of excess levels of the major mycotoxins by standards and enforced regulation. However, many countries where the risk is particularly high have neither the technologies nor the infrastructures to reduce mycotoxin exposure. Moreover, suboptimal field practices and poor storage conditions make the crops vulnerable to fungal infection and subsequent mycotoxin accumulation (Williams, 2008). Maize and groundnuts, two crops susceptible to mycotoxin contamination, are staples in the diets of many people in Africa, Asia, and Latin America. Therefore, interventions should focus on reducing mycotoxin risk in simple and cost-effective ways.

It is important to remember, however, that public health interventions must be readily accepted by their target populations to have any meaningful impact, and must have financial and infrastructural support to be feasible in the parts of the world where they are most needed (Wu and Khlangwiset, 2010b). A critical component to implementing any or all of these methods is public education (Wild and Gong, 2010). Educational efforts should include not only how to use the intervention properly to achieve maximum benefit for mycotoxin risk reduction but also why the interventions are important from the perspectives of public health and food markets, so that users have incentives to continue with the interventions.

Public and governmental education on mycotoxin risk is crucial to provide incentives to adopt interventions. Even if an intervention to reduce mycotoxin risk is cost effective in terms of lives saved and improved quality of life (Wu and Khlangwiset, 2010a), no incentive to implement it may exist unless the effects of mycotoxins on public health and food markets are fully understood. It is worth noting that in Ghana aflatoxin exposure has been shown to be significantly correlated with farmers' knowledge of aflatoxin risk (Jolly et al., 2006), whereas in Benin farmers' knowledge of aflatoxin risk has been shown to be correlated with the motivation to implement interventions to reduce aflatoxin formation (Jolly et al., 2009).

Education must take place on at least three different levels. First, government policy-makers must receive information about the burden of mycotoxin-induced disease in their countries – in terms of effects on both public health and food markets – as well as information about possible interventions, their cost-effectiveness in reducing mycotoxins, and their technical feasibility requirements. Supplying the appropriate information will be one step in sensitizing governments to provide the finances and other resources necessary to initiate the interventions. Second, depending on the intervention characteristics, the farmers, the consumers, or both these groups must receive education on why mycotoxins are a concern and how to implement the intervention in question. Third, international health and agricultural organizations must be informed about the extent to which mycotoxins can affect both food markets and public health. This will provide incentives to aid countries in which foodborne mycotoxins are still a significant public health risk.

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CHAPTER 7