Editorial

Mycotoxins – Food Safety Aspects Euro-Maghrebin Symposium

Production of safe food and drinking water is important to ensure consumers' health. Food-borne diseases are among the most widespread health problems in the developed world. They may be of infectious origin, *i.e.* microbiological, such as Salmonella, Listeria and others, or associated with the consumption of toxic products, *i. e.* natural toxins such as mycotoxins, phycotoxins or chemicals such as pesticides, heavy metals or additives. In several countries these contaminants are subject to legislation that is based on the establishment of an acceptable (or tolerable) daily intake (ADI or TDI). ADI or TDI are estimates of the amount of a food additive that can be ingested daily over a lifetime without appreciable risk to health. Hazard characterization involves evaluation of all available data in order to establish the relationship between dose and response. The dose-response relationship may allow identification of a dose level that is without effect (*e.g.* for chemicals) or estimation of the risk of disease associated with different doses (*e.g.* for microbiological agents and cancer-causing chemicals). A judgement is made concerning the adverse effect that is of most relevance to humans and the highest dose at which the effect does not occur under experimental conditions (no observed adverse effect level or NOAEL). The NOAEL is divided by a safety factor to allow for the uncertainties involved in the extrapolation of the results obtained under experimental conditions to a level of intake that is considered safe for the entire human population. To define the maximal residue limit (MRL), the exposure assessment is taken into account [1].

In September 2005, the Laboratory of Agronomy and Food Safety of the Faculty of Sciences Dhar El Mehraz in Morocco organized in Fez (Morocco) the Euro-Maghrebin Symposium on "*Biological, Chemical Contaminants and Food Safety*" in collaboration with the High School of Technology of Fez (Morocco), the National High School of Agronomy in Toulouse (Laboratory of Chemical Engineering; UMR CNRS/INPT/UPS 5503, France) and the Faculty of Biological, Agricultural and Environmental Engineering in Brussels (Laboratory of the Brewery and Food Industry, Belgium) in the context of joint projects between Morocco and Belgium (PIC, CUD) as well as Morocco and France (AI 03/81). The Symposium was sponsored by *Thermo Electron* (France), *AIR Liquide* (Meknès, Morocco), *Le domaine de Douiet* (Fez, Morocco) and *Bayer Crop Sciences* (Lyon, France).

The aims of this symposium were to:

(i) review the recent research developments in the field of food safety, including pathogenic microorganisms, mycotoxins, pesticides, heavy metals and, more generally, chemical contaminants and risk management;

(ii) strengthen North-South cooperation in scientific research and exchanging experiences and

(iii) to inform the professional sector about the problem of hygiene and food safety with the objective of defining strategies to improve food quality and safety.

The majority of the contributions were on mycotoxins and toxigenic fungi, thus this Special Issue covers several aspects of mycotoxins in the food chain: (i) sampling and analysis, (ii) biosynthesis, (iii) survey of contamination (cereal, olive), (iv) human exposure (in the Balkans and Czech Republic), (v) toxicity, and (vi) decontamination methods. The last three manuscripts cover some bacterial safety aspects which are very important in Southern countries.

A number of cereal and other crops are susceptible to fungal attack either in the field or during storage. These fungi may produce mycotoxins as secondary metabolites. There can be wide and yearly fluctuations in the amount of mycotoxins in foods, depending on many factors such as adverse conditions favouring fungal invasion and growth either in the field or during storage. Although there are geographic and climatic differences in the production and occurrence of mycotoxins, exposure to these substances is worldwide. Mycotoxins may render food unsafe for humans, resulting in acute poisoning episodes (WHO mission report on the outbreak of acute jaundice syndrome in Makueni, Kitui, Thika and Mbeere districts in Kenya, Nairobi, May 27–June 18, 2004) but also long-term disease such as cancer [2]. They are also responsible for economic losses, either directly by impairment of cereal production and quality of the cereal (less vitamins and proteins), or indirectly as mycotoxins can cause death or disease of farm animals. They can affect many target organs and systems, notably the liver (aflatoxin), the kidneys (ochratoxin A; citrinin; fumonisin), and the nervous system (trichothecene, alkaloid). They also exhibit developmental, immune and hormonal effects. Chronic exposure to small amounts of mycotoxins is usually of greater concern than acute toxicity since some of these substances are very potent carcinogens (aflatoxins, ochratoxin, fumonisin). Understanding the mechanism of mycotoxin action in the host animal at the cellular and biochemical level is important in the overall goal to treat or inhibit the action of mycotoxins, thereby potentially controlling illness and deaths attributable to them. Although these toxicants can never be completely removed from the food supply and moreover are very stable during food processing, it is possible through analysis, based on sound scientific knowledge, to define levels in food (tolerances, guideline levels, maximum residue levels) that are unlikely to be of risk to health. This will aid in the harmonization of mycotoxin regulations and control procedures and facilitate international food trade. Knowledge concerning formation, persistence and toxicity of mycotoxins would also help to define new strategies for decontamination and detoxification [2, 3].

Surveillance of the occurrence and prevalence of mycotoxins in food commodities is the basis for exposure assessment and contri-

butes to hazard characterization. Testing for mycotoxins involves obtaining an adequate sample (most important step because of the heterogeneous distribution of mycotoxin in food, especially cereals), preparing the sample, and finally conducting the analytical procedure. The two first manuscripts present the difficulties of these very important tasks. Michel Blanc, an FAO expert, describes sampling protocols. The sampling operation is often the main source of error when assessing the sanitary quality of a lot of agricultural commodities, thus entailing both commercial (downgrading of the product) and sanitary (marketing of a product which poses a health risk for the consumer) consequences. The European Union has recently published several regulations setting very low limits for a given number of food contaminants (pesticides, mycotoxins, heavy metals) in many agricultural products (*e.g.* cereals, oilseeds, dry fruits, coffee, spices). Up to now, most of the sampling standards were established to determine the commercial quality of some products (cereals, oilseeds). This commercial quality is generally related to the contents of proteins, oils and water, which are quoted in % and are relatively homogeneously distributed in the food. This is not the case for mycotoxins which are found in the ppm to ppb range and are heterogeneously distributed. It is also worth noting that sampling procedures set out in the directives, particularly those related to the detection of mycotoxins (aflatoxins and ochratoxin A), are totally inappropriate for sampling products that are stored and delivered in bulk. A new directive (Commission Regulation EU 401/2006) concerning sampling takes into account a major weakness of the previous one: Commission Regulation 401/2006 replaced Directives 98/53/EC, 2002/26/EC, 2003/78/EC and 2005/38/EC on March 29, 2006. It brings together the sampling methods and performance criteria for the methods of analysis to be used for the official controls of all mycotoxins in one single regulation.

The samples must then be submitted for extraction of the toxin and clean up to discard as many interfering substances as possible. The present trend in analysis of mycotoxins is to use an immunoaffinity column (IAC) as the clean-up and enrichment technique. The Association of Analytical Communities (AOAC) and EU have validated several methods which address only a few food commodities. There is therefore a tendency to extrapolate the use of AOAC- or EU-validated methods to the analysis of mycotoxins in other commodities. However, all the tests that are necessary to validate the method are not always performed for each new matrix. Castegnaro *et al.* demonstrate some drawbacks related to the extrapolation of the use of IAC methods which had already been validated for the analysis of three toxins (fumonisin, ochratoxin A and aflatoxins) in one or two matrices to the analysis of the same toxins in very complex matrices. The presence of some components extracted from the matrix leads to underestimation of the three mycotoxins. In addition, in case of ochratoxin A and aflatoxins, extraction under alkaline conditions induces opening of the lactone ring, the latter not being recognised by the antibodies of the IAC, so an underestimation is observed.

In many cases, mycotoxins are formed in the field during the growing season (notably *Fusaria*); however, they can be formed or increase during harvest, drying (*e.g.* ochratoxin A in coffee or cocoa) and storage (*e.g.* ochratoxin A, citrinin, aflatoxin in cereal, nuts and fruits). Mycotoxins are produced by diverse species. Formation of mycotoxins depends on ecological factors such as temperature (Hajjaji *et al.*) but also on the expression of specific polyketid synthase (PKS). The relation between PKS of *Aspergillus ochraceus* and *Aspergillus carbonarius,* and the production of ochratoxin A and/or penicillic acid are described by Atoui *et al.*

Aspergillus flavus and *Aspergillus niger* isolated from olive produce aflatoxin and ochratoxin A, respectively. Roussos *et al.* demonstrate that the toxins could be transferred to the oil. These data are of particular interest, since El Adlouni *et al.* report the presence of aflatoxin B, ochratoxin A and citrinin in olives from Morocco.

It is generally known that the dietary exposure of humans to mycotoxins can be estimated either by analyzing mycotoxins in food using the known amount of the consumed diet or by analyzing levels of mycotoxins in a biological material (serum, urine, kidneys *etc.*). Two manuscripts deal with the correlation between the presence of mycotoxins in food and biomarker of exposure. At present, in the Czech Republic the levels of mycotoxins (aflatoxins B_1, B_2, G_1, G_2, M_1; ochratoxin A, patulin, and deoxynivalenol) in foodstuffs (pistachio, cereals, flour, malt, milk, fruits) of home-grown products are relatively low. However, the overall situation may change due to the globalization of the food market (Malir *et al.*). In the same manuscript the authors report the analysis of ochratoxin A (OTA) blood concentration and aflotoxin M_1 in urine.

Currently OTA is the most probable mycotoxin involved in the Balkan nephropathy (BEN) endemic and some renal tumours could also be involved in the same pathologies in other European countries [4]. This toxin is responsible for nephrotoxicity in swine, notably in Denmark, and decreased productivity in pig and poultry farming. In order to confirm the exposure of the population to OTA in the Vratza district in Bulgaria where there is a high incidence of BEN/UTT, and to establish if a correlation between OTA food intake, OTA blood concentration and urinary OTA excretion, Castegnaro *et al.* followed some individuals over one month. In parallel, the same parameters (blood concentration, OTA excretion and DNA adduct in relation to OTA food intake) in rats fed with increasing amounts of OTA for one month were analysed. The data confirm those of previous studies in humans in this area of the Balkans, in that some inhabitants have very high exposure to OTA which is, in part, reflected by high levels in blood and urine. However, there is no direct correlation between OTA in the blood or urine and OTA consumed; thus OTA in the blood or urine cannot be recommended as a biomarker of OTA exposure. OTA DNA adducts have been found in the kidney (which is the target organ of animals treated with OTA) and in humans suffering from nephropathy and urothelial tract tumours, who have a high consumption of OTA, and high OTA plasma and tissue concentrations.

Renal OTA carcinogenicity has been clearly demonstrated in rodent species at doses exceeding those inducing nephrotoxicity [5–7]. This observation together with the lowest observed effect level for nephrotoxicity in swine (found to be the most sensitive animal species) were used for safety evaluation and the establishment of the provisional tolerable daily intake varying between 1.2–5.7 ng/kg bw/day ([8] although the JECFA in 2001 retained its previously established provisional weekly intake of 100 ng/kg bw/day [9]). Previously, the International Agency for Research on Cancer [5] classified OTA as a possible human carcinogen (group 2B), based on sufficient evidence for carcinogenicity in animals, but inadequate evidence in humans. Convincing evidence indicates

that exposure to OTA results in DNA damage in the kidney, liver and testis. The mechanisms by which OTA is carcinogenic are not entirely elucidated and two hypotheses are still under discussion: (i) an indirect mechanism which would result in a classification as an epigenetic carcinogen [10, 11] or (ii) due to direct covalent binding of OTA on DNA, suggesting genotoxic mechanisms being involved in the carcinogenicity [12, 13]. Although there is evidence for a time- and dose-dependent induction of DNA lesions *in vivo* when applying the ^{32}P-postlabelling technique, the chemical identities of adducts and metabolites implicated need to be elucidated [14]. Faucet *et al.* confirm that the main pathway involved is an OTA quinone pathway.

Although agronomic and other practices are aimed at decreasing or eliminating fungi and/or mycotoxins in the field, there is still good reason to look at post-harvest means to eliminate or inactivate mycotoxins in grains and other commodities. These detoxification strategies can be grouped into three categories: physical, chemical and biological methods. A major interest in controlling the effects of mycotoxins in animals has been in developing binders of mycotoxins to render them unavailable for absorption by the host animal. Biological detoxification is the method of choice to deactivate mycotoxins. This comprises binding by adsorptive materials as well as microbial inactivation by specific micro-organisms or enzymes (Schatzmayr *et al*.).

The paper by Jawich *et al.* analyses the impact of penconazole (an antifungal compound) on the viability of yeasts used in the wine-making process.

Recent data from developing and developed countries indicate that at least 10% of consumers may experience a foodborne disease once in their lives. In Europe, monitoring the impact of foodborne disease mortality is not a major priority; however, 50 000 cases of acute gastro-enteritis *per* million inhabitants annually are reported. In France, 559 cases of foodborne diseases were reported in 2001, of which 64% were due to Salmonellosis. In Morocco, although efforts have been made to improve food safety and quality, foodborne diseases still represent one of the main causes of mortality. Bacterial foodborne diseases are caused by red meat and meat products and 14.7% occurred in the city of Casablanca. Cohen *et al*. analyse not only the incidence and/or levels of pathogenic and non-pathogenic microorganisms present in red meat and beef offal produced in Casablanca, but also the effect of seasonality and distribution location on the incidence levels of such microorganisms.

The poultry industry has to deal with the problem of the transfer of pathogens (*e.g. Salmonella, Campylobacter*) from chickens to humans. For many decades antibiotic growth promoters have been used in feed for farm animals. Because of the general problem of increased resistance of bacteria and the decreasing acceptance of the consumers for this type of additive, antibiotics have already been banned in some countries and will not have a future in the EU. Klose *et al*. describe the interest of probiotic strains (*Pediococcus acidilactici, Enterococcus faecium, Bifidobacterium animalis* ssp. *animalis, Lactobacillus reuteri, L. salivarius* ssp. *salivarius*) as food additives for chickens.

The application of vacuum packaging followed by in-pack processing offers a new variety of cook-chilled products which are in high demand. In comparison with conventional processing, it offers shelf-life extension and improved eating quality. However, the use of vacuum, which would inhibit the usual competitive aerobic spoilage microflora, together with the longer shelf life, might allow the proliferation of those facultative anaerobes (*e.g. Staphylococcus aureus*) surviving the cooking/pasteurization step. The great concern of the food industry to offer the best sensory quality has led to the design of minimal heat treatments which should be questioned in terms of pasteurization, as pasteurized foods are responsible for numerous foodborne outbreaks. Hassani *et al.* demonstrate that exposure of microorganisms to sublethal temperatures for short periods of time might act as heat shocks, increasing microbial heat resistance to a subsequent heat treatment, and also developed a mathematical model.

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