

CHAPTER 1

RISK ANALYSIS OF MYCOTOXINS WITH PARTICULAR EMPHASIS IN OCHRATOXIN A

Consumers are taking unprecedented interest in the way food is produced, processed and marketed and are increasingly calling for their governments to accept greater responsibility for food safety and consumer protection (FAO/WHO, 2003). There is growing concern for ways in assessing possible preventive and corrective actions in order to develop new practices to reduce or eliminate different hazards occurring in food. To facilitate the prevention approach, information on risk analysis is needed. Risk analysis is an interactive process that considers risk assessment, risk management and risk communication (Figure 1). It is important that there is interaction between these major areas.

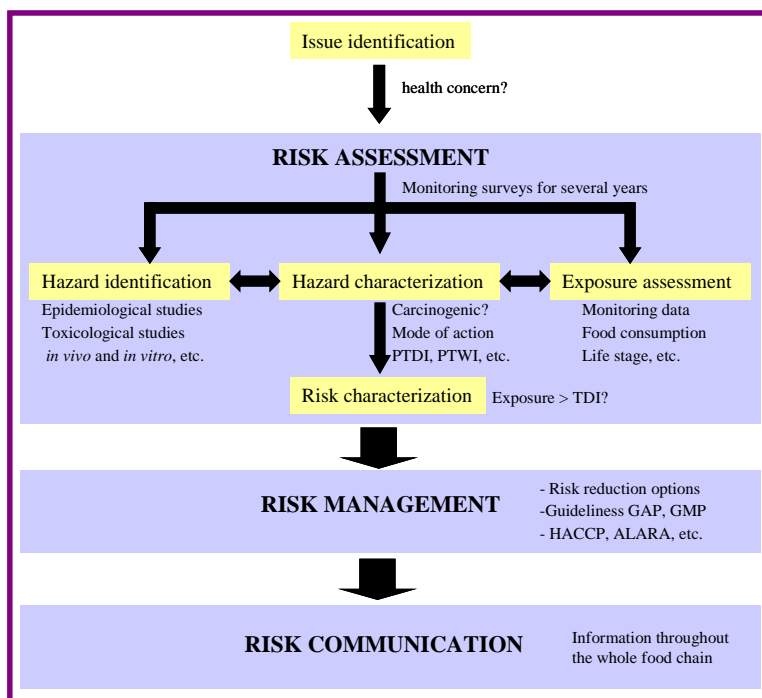


Figure 1. Interactive process of risk analysis of mycotoxins in foods (modified from Kuiper-Goodman, 2004).

Before completing the definition of risk analysis and focusing it on the problematic of mycotoxins in food, especially on ochratoxin A (OTA) in grapes and wine, the topic of this manuscript, a couple of definitions are needed:

- **Hazard** is defined as the intrinsic property of a biological, chemical or physical agent to cause adverse health effects under specific conditions.

- **Risk** is defined as an estimate of the probability of the occurrence of an adverse health effect in humans, weighted for its severity, that may result from exposure to a biological, chemical or physical agent in food.

1.1. RISK ASSESSMENT

Risk assessment is the result of the identification and characterization of the food hazard and an exposure evaluation, and mainly consists in the following aspects:

1.1.1. Hazard identification

Hazard identification is the qualitative indication that a contaminant can cause adverse effects on health, involving a complete toxicological and epidemiological assessment.

The hazard object to study in the present manuscript is OTA, especially in grapes and grape-derivatives such as wine. The main characteristics of OTA, the evaluation of the toxic potency of this mycotoxin, the dosage at which it exhibits toxicity, the identity of the recipient, and the route by which it is introduced to the recipient is showed in part of the following review (see 6.3.4.2.), as well as in the chapter 5 of this thesis.

- **Review: Ochratoxin A (OTA) in wines, musts and grape juices: occurrence, regulations and methods of analysis.** *Food Sci. Technol. Int.* **8**, 325-335 (2002).

1.1.2. Hazard characterization

Hazard characterization can be described as a qualitative and quantitative evaluation of the nature of the adverse effects, that is to say, an extrapolation phase to humans of the dose response and hazard exposition.

The evaluation of toxicological data normally results in the estimation of a Provisional Tolerable Weekly Intake (PTWI) or a Provisional Tolerable Daily Intake (PTDI), and the ones for OTA are mentioned in chapter 5.

1.1.3. Exposure assessment

Dietary exposure to OTA can be assessed by combining data on concentration in all food products with data on their consumption. In the European Union (EU), efforts to assess exposure are undertaken within Scientific Cooperation on Questions relating to Food projects (SCOOP), funded by the European Commission. The SCOOP projects are targeted to make the best estimates of intake of several mycotoxins by EU inhabitants. For the intake assessment two types of information are needed, how much food containing the contaminant is consumed daily and what is the level of contamination.

Data on the levels of OTA found both in foods (see 5.3.6.) and in human tissues (see 5.3.7.) would be required in order to demonstrate the real risk of the OTA exposure. In particular, a study of the occurrence of OTA in Spanish wines was carried out and could contribute in some way to the exposure assessment of this mycotoxin, and the results are summarised in the following paper (see 6.4.3.):

- Ochratoxin A in wines, musts and grape juices from Spain. *J. Sci. Food Agric.* 84, 591-594 (2004).

1.1.4. Risk characterization

Risk characterization involves a comparison of the exposure data and the doses that could cause the hazard. If exposure exceeds the intake for which the risk is considered to be insignificant, recommendations for risk reduction may be made.

Prevention, elimination and reduction procedures to acceptable levels of OTA, as well as information about the most effective intervention strategies are given in several chapters of this thesis (see 5.4.; 6.5.; 9).

In reality, reducing an exposure to zero is not always possible. The best way to prevent this risk from increasing is to keep one's exposure As Low As Reasonably Achievable (ALARA). The ALARA level, which may be viewed as the irreducible level for a contaminant, is defined as the concentration of a substance that cannot be eliminated from a food without involving the discard of that food altogether or without severely compromising the availability of major food supplies. This covers the case of the Joint Food and Agriculture Organization of the United Nations (FAO)/World Health

Organization (WHO) Expert Committee on Food Additives (JECFA) evaluations of the aflatoxins made in 1987 and 1997 (JECFA, 1999), and in 1990 and 1995 JECFA have also evaluated the risk of other mycotoxins such as OTA (Olsen, 1996).

1.2. RISK MANAGEMENT

Consumers expect protection from hazards occurring along the entire food chain, from primary producer through consumer, often described as *farm-to-table continuum* or *farm to fork approach*. Protection will only occur if all sectors in the chain operate in an integrated way, and food control systems address all stages of this chain (FAO/WHO, 2003). The introduction of preventive approaches such as the food management system Hazard Analysis Critical Control Point (HACCP), improves the consumer protection, effectively stimulates agriculture and the food processing industry, and promotes domestic and international food trade by implementing regular and systematic controls to anticipate the problems of food safety. The HACCP system itself, although well established in food processing and manufacture world-wide, is not at present widely used in primary food production. Since HACCP has demonstrated its effectiveness, the EU is encouraging projects which aim to apply this method to the control of mycotoxins. Thus, the safe management of OTA in wine-grape production could be analysed according to this system.

The HACCP approach involves the fulfilment of seven clearly defined stages or principles:

1. Risk assessment and description of control measures.

It consists in identify the significant hazards and risks through the stages of the manufacturing process of the product, and describe the appropriate preventive measures.

2. Determination of the critical control points (CCPs).

The critical control points are stages in the production process where control is possible, and with an absence of supervision of which, may lead to a health risk.

3. Establishment of critical limits.

The critical limits are quantitative parameters which will ensure that control is maintained at each CCP. The parameters selected always include a margin of safety.

4. Design of control procedures for the CCPs.

Monitoring procedures should be accurate and rapid.

5. Establishment of corrective actions.

For each CCP, corrective action must be provided for the case where monitoring reveals that critical limits are not being achieved and therefore there is the presence of a risk.

6. Establishment of verification procedures.

Checking the entire HACCP system to ensure that appropriate controls are in place is needed. In the case of mycotoxins, this phase also requires a method to analyse them. If levels of mycotoxins exceeding the required limit are found, immediate action must be provided to determine where the control is defective. This may lead to the establishment of other CCPs or the modification of the critical limits imposed in the existing CCP.

7. Documentation and record keeping.

Detailed documentation of all the stages in the HACCP plan is indispensable and should contain full information on mycotoxins, their risks and the conditions in which they are formed. Information on control procedures and corrective actions should be also documented.

Other quality assurance systems applied to prevent mycotoxins are the establishment of Good Agricultural Practices (GAP) and Good Manufacturing Practices (GMP). Building linkages between surveillance, monitoring and control programs, coordination of work with government, private and consumer sectors and increasing awareness of decision makers are other strategies focused to the same aim.

By elucidating the mycoflora of foods, mycotoxin hazards might be predicted and the appropriate control measures undertaken. Fungi responsible for OTA contamination in Spanish grapes, their dynamics in the vineyard and factors which influence their growth and metabolism in the field were studied from 2001 to 2004 (see 7.8.):

- Occurrence of ochratoxin A and toxigenic potential of fungal isolates from Spanish grapes. *J. Sci. Food Agric.* 84, 541-546 (2004).

- Ochratoxin A-producing fungi in Spanish wine grapes and their relationship with meteorological conditions. *Eur. J. Plant Pathol.* (in press).

and results were revised together with data from other Spanish vineyards:

- Mycobiota and ochratoxin A producing fungi from Spanish wine grapes. *Int. J. Food Microbiol.* (in press).

Furthermore, accurate information on the impact of the key environmental determinants and the relationship between these key factors were also studied, in order to understand the ecological role of ochratoxigenic fungi in vineyards. Emphasis was placed in finding the marginal and optimum conditions for growth and OTA production (see 8.4.2).

(.../...)

- Influence of water activity and temperature on growth of isolates of *Aspergillus* section *Nigri* obtained from grapes. *In. J. Food Microbiol.* **96**, 19-27 (2004).

- *Aspergillus carbonarius* growth and ochratoxin A production on a synthetic grape medium in relation to environmental factors. *J. Appl. Microbiol.* **98**, 839-844 (2005).

- Skin damage, high temperature and relative humidity as detrimental factors for *Aspergillus carbonarius* infection and ochratoxin A production in grapes. *Food Contr.* (submitted).

- Incubation time and water activity effects on ochratoxin A production by *Aspergillus* section *Nigri* strains isolated from grapes. *Lett. Appl. Microbiol.* **38**, 72-77 (2004).

- Kinetics of ochratoxin A production and accumulation by *Aspergillus carbonarius* on synthetic grape medium at different temperature levels. *J. Food Sci.* (submitted).

- Effect of photoperiod and day-night temperatures simulating field conditions on growth and ochratoxin A production of *Aspergillus carbonarius* strains isolated from grapes. *Food Microbiol.* (in press).

and a review of the ecophysiological studies was carried out (see 8.4.2.1.):

- Water relations of germination, growth and ochratoxin A production by European isolates of *Aspergillus carbonarius* from wine and table grapes. *Int. J. Food Microbiol.* (in press).

Effective vine protection systems such as the application of fungicides were also studied (see 8.4.2.4).

- Impact of fungicides on *Aspergillus carbonarius* growth and ochratoxin A production on synthetic grape-like medium and on grapes. *Food Addit. Contam.* (submitted).

- Effect of chemical treatments on ochratoxigenic fungi and common mycobiota of grapes (*Vitis vinifera*). *J. Food Protection* (submitted).

Finally, a preliminar approach of monitoring systems for moulds invading grapes was carried out (see 8.5.).

(.../...)

- Visualisation of grape (*Vitis vinifera*) infection using transgenic green fluorescence protein (GFP) strains of *Aspergillus carbonarius*. *FEMS Microbiology Letters* (submitted).

1.3. RISK COMMUNICATION

Risk communication is the interactive exchange of information and opinions concerning hazards and risks, risk related factors and risk perceptions, throughout the risk analysis process: risk assessors, risk managers, consumers, industry, the academic community and other interested parties. It includes the communication of risk assessment findings and the basis of risk management decisions. These activities comprise the provision of balanced factual information to consumers; the provision of information packages and educational programmes for key officials and workers in the food industry; development of train-the-trainer programmes; and provision of reference literature to extension workers in the agriculture and health sectors (FAO/WHO, 2003).

Continuous risk communication, involving the effective measures for preventing, reducing or eliminating the risk, is as important as punctual communication, done in the cases of food safety crisis. International organizations such as FAO, WHO, United Nations Environment Programme (UNEP), etc. system are engaged in providing essential information on various aspects of prevention and control of mycotoxin problems to all the countries. Further information tasks should be started and administered by the government of each country through ministries and organizations. Conferences, symposiums, trainings and workshops on current informations of mycotoxins should be promoted.

The possible risk attributable to OTA must be widely disseminated, as should the existing methods for preventing them. A small contribution of the present work in the OTA-risk communication was the publication of a chapter in a book that was distributed among several farmers of Spain and Portugal (see appendix 3), together with the recent participation in a workshop carried out in Sicily (20th-21st October 2005), where apart from several conferences by prestigious researchers, triptychs suggesting preventive and control measures of OTA in grapes and wine were distributed.

▪ **Primera aproximación al control de la ocratoxina A en vinos. Estudios micológicos.** In: *Impacto da contaminação fúngica sobre a competitividade de vinhos. Ocratoxina A*. Cerdeira, A., Venâncio, A., Alves, A., Barros, P. and Simões, T. (eds.). Micoteca da Universidade do Minho. Braga, Portugal, 29-52 (2003).

The emerging problems regarding food poisoning pose an universal importance which should be considered with an unified approach of the world-wide countries. Impediments to a globally harmonized approach to risk analysis relate in part to data interpretation and analysis and differences in food intake patterns between countries (Kuiper-Goodman, 2004).

1.4. REFERENCES

FAO/WHO, Food and Agriculture Organization of the United Nations/World Health Organization. 2003. Assuring food safety and quality: guidelines for strengthening national food control systems. *FAO Food and nutrition paper 76*, Rome, Italy.

JECFA, Joint FAO/WHO Expert Committee on Food Additives. 1997. Safety evaluation of certain food additives and contaminants. 49th meeting. *World Health Organization, food additives series 40*, Geneva, Switzerland, 359-468.

JECFA, Joint FAO/WHO Expert Committee on Food Additives. 1999. Evaluation of certain food additives and contaminants. Aflatoxins and recommendations. *World Health Organization, technical report series 884*, Geneva, Switzerland, 69-80.

Kuiper-Goodman, T. 2004. Risk assessment and risk management of mycotoxins in food. In: *Mycotoxins in food, detection and control*. Magan, N. and Olsen, M. (eds.). Woodhead Publishing Ltd., Cambridge, UK, 6.

Olsen, M. 1996. Toxicological evaluation of certain food additives and contaminants, ochratoxin A. *World Health Organization, food additives series 35*, Geneva, Switzerland, 365-376.