ILSI EUROPE CONCISE MONOGRAPH SERIES

# PRINCIPLES OF RISK ASSESSMENT OF FOOD AND DRINKING WATER RELATED TO HUMAN HEALTH

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# PRINCIPLES OF RISK ASSESSMENT OF FOOD AND DRINKING WATER RELATED TO HUMAN HEALTH

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# FOREWORD

In the last five years the term Risk Assessment has been used probably more often in relation to safety of food and chemicals than any other word.

Indeed, the need to attain a more systematic approach, among others in setting standards to assure safety, is evident since calamities have occurred regularly and will, most probably, also happen in the future.

The Risk Assessment Paradigm consisting of four steps to characterise a risk will fulfil such a systematic approach when each step is described in detail, critically evaluated, ameliorated where possible, and made accessible to a broad audience.

This concise monograph aims to describe the state of the art in the Risk Assessment Process. Importantly, it considers biological and chemical agents and aims to integrate chemical and microbial risk assessment wherever appropriate and feasible. Parallels with and specific aspects of microbiological risk assessment are highlighted. Case studies are used to illustrate each risk assessment step. Current needs for improvements in exposure assessment and hazard characterisation are described.

Quantitative Risk Assessment will also be increasingly necessary to better characterise increasingly accepted risks, and to compare these to other common existing risks.

Risk Assessment should however not be seen as a separate entity but should be considered as the scientific part of the total Risk Analysis process, where Risk Management, Risk Communication and Risk Perception are additional important issues, e.g. Risk Assessment is a supportive and necessary tool for Risk Management.

This concise monograph is intended to be a source for public health professionals, regulatory authorities, as well as for scientists interested and involved in risk evaluation procedures.

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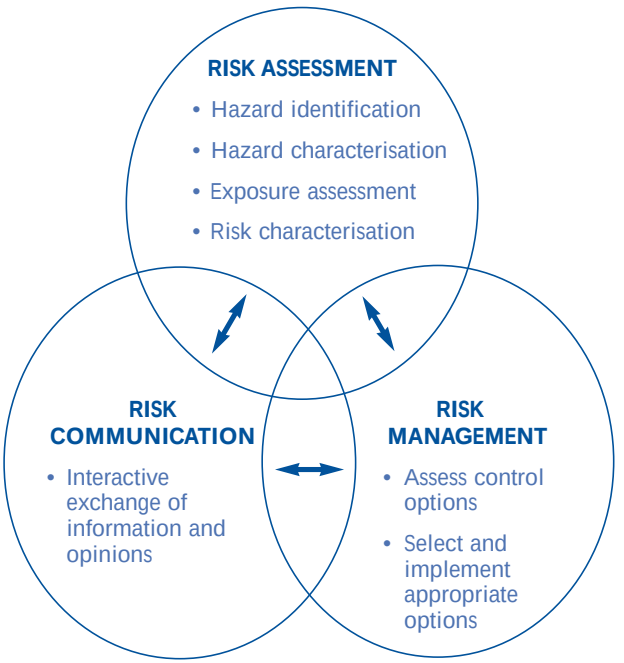
# INTRODUCTION

Safe production of food and drinking water is an important issue and subject of legislation in most countries. Legislation governs the establishment of safe levels of chemical substances to be allowed in food and drinking water, whether occurring naturally, as deliberate additions or as contaminants. It also defines hygiene measures to minimise spread of pathogenic agents and the diseases that they may cause. Despite these measures, foodborne illnesses are among the most widespread health problems in the developed world. They may be caused by toxic (chemical) or infectious (microbiological) agents entering the body during ingestion of contaminated food or water. To ensure that resources are used most effectively, it is essential to have a sound basis for assessing the risks of the many different chemical and microbiological agents that may be ingested via food and water. There are other important issues in food safety, including transmissible spongiform encephalopathies (the variant Creutzfeldt Jacobs Disease associated with bovine spongiform encephalopathy (BSE), so-called 'mad-cow disease') and concern over perceived risk associated with genetically modified (GM) organisms. However, these will not be discussed in this monograph.

It is generally agreed that risk assessment should be an independent scientific process, distinct from measures taken to control and manage the risk. The overall risk analysis process includes risk assessment, risk management and risk communication, and involves political, social economic and technical considerations. It also needs to take into account the public's perception of risk (Figure 1). Risk management strategies may be regulatory, advisory or technological and take into account factors such as the size of the exposed population, resources required and available, costs of implementation and the scientific quality and certainty of the risk assessment. Risk communication should include interactive exchange of information and opinions among risk assessors, risk managers, consumers and all other interested parties, often called stakeholders. Whilst there is much similarity in the scientific risk assessment for food and for water, and for chemical and microbiological agents therein, the

**FIGURE 1**
**Risk analysis framework**




Risk assessment and management also need to take into account the public's perceptions of the acceptability of risk in different situations.

ensuing risk management strategies will differ, and it is not appropriate to consider risk management and communication in detail here.

This concise monograph describes the approaches taken in establishing whether consumption of food or drinking water contaminated with certain chemicals or microorganisms may pose a risk to human health. It aims to provide the non-expert with an appreciation of the scientific knowledge and uncertainties involved in the process of risk assessment. Risk assessment may be defined as "*identification and quantification of the risk resulting from a specific use or occurrence of a chemical, physical or microbiological agent, taking into account possible harmful effects on individual people or society of using the agent in the amount and manner proposed and all the possible routes of exposure*". It is the first stage of a process referred to as risk analysis and the underlying objective of risk assessment is to provide the scientific basis for control and management of risk.

This concise monograph focuses specifically on risk assessment for human health. There is increasing recognition of the potential for chemical and microbiological agents to have adverse effects on the environment and on wild life, and these may ultimately also have implications for human health. There is also a move towards integration of human and environmental risk assessment. This includes modelling of potential levels of contaminants in different media (e.g. soil, groundwater) and subsequent transfer into the food chain to evaluate the risk to human health and the environment. Use of comparable methodology would facilitate assessment of relative risks and prioritisation of risk management procedures. However, it is beyond the scope of this concise monograph to consider ecological risk assessment.

Risk is defined as the chance or probability of an adverse health effect occurring and the severity of that effect. It could be expected to have a numerical value, allowing different risks to be compared and placed in relative order of likelihood. This is frequently the case for risks of accidents, for example, of death from road accidents, compiled from statistical data of the occurrence of such events. In contrast, quantification of risks due to chemicals or microorganisms is rarely feasible for several reasons.

First, it may not always be possible to establish conclusive links between cause and effect, this is particularly the case for chemical agents, but sometimes also for microbiological ones. Modern scientific knowledge has allowed us to identify and control many of the most harmful infective agents that were responsible for life-threatening diseases in previous generations. Thus, widespread chlorination of drinking water led to virtual eradication of diseases such as cholera and typhoid in the developed world. Causal links with a specific agent (or group of agents) have not been established for all modern diseases. Mankind is exposed, usually at low levels, to an increasing number of synthetic chemicals in addition to those that occur in nature, and it is rarely possible to identify human health problems arising from specific chemical substances except in those cases where high exposure is documented. Establishing a link between microbial agents and illness is more direct than for chemicals because the effects are acute and the microorganism can frequently be isolated from the patient. However, new strains of a microorganism may arise with differing capacity to cause disease. Even where a relationship with disease can be established, the causal agent may derive from many different sources, some of which may be difficult to identify and quantify. Finally, differences in human susceptibility mean that if two individuals are exposed to the same dose of a harmful agent, under

similar circumstances, they will not inevitably respond in the same way or with the same degree of severity. This means that risks may need to be quantified differently for persons with different degrees of susceptibility. In common language, risk often has a perceptual value, but risk assessment deals only with scientific facts, and not with emotional influences.

Thus, risk assessment for chemical and microbiological agents requires consideration of the factors alluded to in the previous paragraph, and these are generally encompassed within the stages of the overall risk assessment process, defined as:

- Hazard identification.
- Hazard characterisation.
- Exposure assessment.
- Risk characterisation.

The following section considers the stages of risk assessment in detail. Many individuals involved in risk assessment deal with either chemical or microbiological agents. Precise definitions of scientific terminology are frequently contentious, and this is no less true in the area of risk assessment. There are some fundamental differences in definitions of specific terms, as will be described in the relevant sections of this monograph, and the similarities in approach for chemical and microbiological agents are frequently not appreciated. This concise monograph therefore highlights the essential similarities and differences in the approaches that are currently used for Microbiological Risk Assessment (MRA) and for Chemical Risk Assessment (CRA), with the aim of increasing transparency and understanding of the overall processes. By way of examples, the different stages are illustrated by reference to:

A. A hypothetical synthetic pesticide used on food crops, assuming that this is a new product that will be subjected to safety testing following the most recent guidelines.

B. Aflatoxins – a group of mycotoxins produced by the mould *Aspergillus flavus* which contaminates maize and groundnuts and their products. There are several different forms of aflatoxin, of which the most common is known as aflatoxin B1. There is a large and convincing amount of evidence that aflatoxins cause liver cancer in humans, particularly in individuals infected with viral hepatitis.

C. *Listeria monocytogenes* – the bacterium responsible for the disease listeriosis, which affects the foetus, the elderly and immunocompromised individuals. The major route of transmission is via foods in which *Listeria monocytogenes* could multiply and reach high levels mostly due to insufficient control of storage conditions (time and temperature), e.g. for extended shelf-life chilled foods.

The process of ensuring the safety of food additives is considered in more detail in another ILSI Europe Concise Monograph: *The Acceptable Daily Intake: A Tool for Ensuring Food Safety*.

# THE RISK ASSESSMENT PROCESS

## Statement of purpose or problem formulation

Risk assessment is a scientific process, conducted by scientific experts, which may begin with a statement of purpose intended to define the reasons that the risk assessment is required and support the aims of the subsequent stages of risk management.

Risk management is defined as *"the process of weighing policy alternatives in the light of the results of risk assessment and, if required, selecting and implementing appropriate control options, including regulatory measures."* It needs to consider social and economic factors in addition to juridical and policy considerations. It is essential for the scientists to have clearly agreed aims and objectives that have been defined taking into account the disparate interests of all possible stakeholders, including the public, agricultural and food processing industries, food retailers, etc. The financial interests of the different stakeholders often conflict, and therefore balancing the different needs is a political process, which also takes into account regional socioeconomic factors, such as employment. Such considerations are kept strictly separate from the risk assessment process in order to assure its objectivity.

Chemical risk assessment often does not have a formal statement of purpose. However, it may be defined implicitly in a generic form, as in the terms of reference of an expert committee, such as the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) (Box 1A). In the context of this monograph, CRA concerns the definition of acceptable or tolerable levels of intake for a chemical in food or water, which may require review and revision in the light of new information. It normally focuses on a single chemical substance, a group of substances, or a defined mixture of substances of interest. Only in very few circumstances does it focus on specific types of food in which the chemicals are contained.

Expert committees may also be asked to answer specific questions relating to naturally occurring substances, or to chemical additives and contaminants that have been in use for many years and not subjected to currently accepted testing strategies. Such questions could then be seen as providing a statement of purpose. The example used here concerns the recent evaluation by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) of aflatoxin (see Box 1B). A full risk assessment may be required to define situations where acceptable/tolerable levels are exceeded.

Unlike the CRA, the MRA may focus on one or all of the different stages of food production, processing, formulation, packaging, storage, distribution, sales and preparation, the overall process sometimes referred to as "farm to fork," in addition to the microbiological agent of interest. The important issue is the level of contamination at the point of consumption and it is not always necessary to conduct risk assessment for the entire route. The risk assessment may be conducted in response to several different stimuli such as:

- outbreak of disease associated with known pathogens;
- public concern about potential occurrence of pathogens;
- concern over emerging, or re-emerging pathogens;
- the need to establish or evaluate control measures, in compliance with international legislation;
- the need to design a safe process.

## BOX 1

### A: The statement of purpose – objectives of JMPR

"… to determine a no-observed-adverse-effect level (NOAEL) based upon consideration of the total toxicology database, which will be utilised in conjunction with an appropriate safety factor to determine the Acceptable Daily Intake"

### B: The statement of purpose – JECFA review of aflatoxin

"… the Committee considered estimates of the carcinogenic potency of aflatoxins and the potential risks associated with their intake"
and
"... the possible impact of applying hypothetical standards to aflatoxin contamination"

### C: The statement of purpose – Quantitative risk assessment of human listeriosis from consumption of soft cheese made from raw milk

(from Bemhah *et al*, Preventative Veterinary Medicine, vol 37, pages 129-145, 1998)
"…the probability of human listeriosis and death associated with the consumption of soft cheese made from unpasteurised milk"

Microorganisms may be eliminated from foods by heat treatments, they may survive in frozen or preserved food, or they may proliferate. The degree of contamination of a food product at the time of consumption varies according to the effects of the different processes and storage on the numbers of microorganisms in the components. In contrast, the amount of a chemical does not normally increase; control of the maximum amount at the source of contamination will control the amount that the consumer ingests.

The statement of purpose includes a description of the product, process, use and the consumers involved, how the output of the risk assessment is intended to be used (e.g. for support of preventive or interventionary measures), and the nature of the input data required. Possible objectives include an estimation of the risk associated with a microbiological hazard in the total food supply to a defined population of consumers, or the risks associated with a microbiological agent in a specific food commodity. Risk assessment of drinking water may need to take into account a number of different microbiological agents, and that there is usually not one single important killing step for all pathogens.

The formal statement of purpose of the MRA is therefore likely to be specific to each situation (e.g. Box 1C) and will influence the information and data requirements. If the focus is on the microbiological agent, information will be required on whether food and / or water are implicated in the aetiology of disease, and which specific food types are involved. If the focus is on food (or water), data are needed on the types of pathogen that could be involved and their growth and behaviour in that medium. The MRA may also operate at a number of different levels depending on intended outcome. At the simplest level, this may be to allow the individual to make a personal choice on what to eat or

drink, based upon the information provided in the risk assessment. At the supplier level, it may be to identify processes that are most likely to present hazards. For food production or preparation, MRA in the food industry supports HACCP (hazard analysis critical control point) as the risk management tool. HACCP is described in detail in the ILSI Europe Concise Monograph: *A Simple Guide to Understanding and Applying the Hazard Analysis Critical Control Point*. Basically, it provides a systematic approach to the assurance of the safety of specific food products by control and monitoring of processes. It involves identification of hazards, such as pathogenic agents and the conditions leading to their elimination, survival or proliferation, during the manufacture, distribution and use of a food product, and definition of measures for their control and/or prevention at the critical points identified. It also includes mechanisms to monitor the effectiveness of the controls and the HACCP process.

The results of the MRA are described in terms of estimated:

- annual occurrence of infection, i.e. how many people in the general population of a country will be infected, with or without symptoms, each year due to consuming a common foodstuff;
- annual rate of illness per 100,000 population, which could be more easily related to a specific subgroup such as children or pregnant women;
- rate of illness per 100,000 servings, allowing frequency of consumption to be taken into account and estimation of risk of infection from a single serving.

## Hazard identification

The emphasis of hazard identification differs for chemical and microbiological agents. As described by Paracelsus nearly 500 years ago, "All substances are poisons; there is none which is not a poison. The right dose differentiates a poison and a remedy." This means that any chemical substance is likely to produce some form of adverse effect if taken in sufficient quantity. Also, a single chemical substance may be associated with several different adverse health effects, not all of which would necessarily be expressed in a specific exposure scenario. Therefore, experts conducting risk assessment of chemical substances define the potential health effects as individual hazards, which need to be considered separately during the evaluation. To the toxicologist, hazard is defined as a "*set of inherent properties of a substance, mixture of substances or process involving substances that make it capable of causing adverse effects to organisms or the environment*" (IUPAC).

In contrast, not all microorganisms are pathogenic, but because pathogens are able to multiply to cause infection, even a very small number have the potential to cause disease. Therefore, microbiological hazard identification focuses primarily on identification of the presence of pathogenic agents rather than the diseases that they cause, as given in the CODEX definition of hazard: *"a biological, chemical or physical agent with the potential to cause an adverse health effect"*. Hence hazards may be bacteria, viruses, yeasts, moulds, algae, parasitic protozoa, their toxins and metabolites and their adverse health effects. Microbiological hazard identification can be considered from the pathogen perspective (which agent to be considered), from the public health perspective (which illnesses in which categories of the population) and from the product perspective. In MRA, emphasis is given to the agent, as this is the aspect that is controlled to prevent an adverse effect. In CRA, emphasis is given to the adverse effects, as will be demonstrated in the following section.

### Chemical hazard identification

Chemical hazard identification has been defined as *"determination of substances of concern, their adverse effects, target populations and conditions of exposure, taking into*

*account toxicity data and knowledge of effects on human health, other organisms and their environment"* (IUPAC). In the context of this concise monograph, the aim is to evaluate whether the chemical has the potential to cause adverse effects in humans based upon review of all available data on toxicity and the biological mechanism that leads to toxicity. Different types of adverse effect may be caused by various chemical substances. In theory, many types of toxicity may result from a single exposure to the chemical agent, and if the effect is seen within a few days of the exposure, then it is referred to as acute. In practice, the chemicals that are found in food and drinking water are unlikely to cause acute effects unless consumed in extremely high quantities. Therefore, toxicity is most likely to result from repeated exposures over extended periods of time, which is known as chronic toxicity.

The data used in hazard identification may include results from many different types of study, including:

- human studies – epidemiology, case reports or volunteer studies;
- toxicity studies conducted in laboratory animals;
- alternative approaches, including use of *in vitro* models such as cell cultures or tissue slices, and comparisons with structurally-related chemical substances.

Each of these sources of information has advantages and limitations with respect to the relevance of the data, and greatest confidence is provided by an assessment that includes a combination of the different approaches. Often, the information provided by different types of studies is complementary, e.g. *in vitro* data provide information on the mode of action of given toxic effects. The actual amount and quality of available data can vary enormously for different types of chemical. Recently introduced synthetic chemicals are likely to have been subjected to a defined toxicity testing strategy (as in Box 2A), following published guidelines and rigorously controlled protocols and reporting procedures. However, there are likely to be few or no human data and little information on the mechanism of toxicity. In contrast, naturally occurring chemicals, and synthetic chemicals that have been in use for many years, will have been tested by less stringent procedures and using incomplete test batteries compared to current requirements, but it is more likely that epidemiology studies will have been reported. It may therefore be possible for the risk assessment to focus on the human data, as in the example on Box 2B. There may also be information available relating to the mechanisms of action and its relevance to humans for chemicals of greatest concern.

### *Human studies*

Epidemiology studies have the advantage of providing information of direct relevance to humans. They can allow for the full range of susceptibility of different individuals (or focus on specific subgroups of individuals). They also investigate the effects of realistic conditions of exposure in the presence of other risk factors, such as smoking. There are two major approaches to epidemiological studies, each with well-established advantages and weaknesses. Descriptive epidemiology aims to explore correlations between the incidence of a disease and assumptions of exposure in populations. It is frequently cross-sectional in design which means that data on the disease and the exposure are generated at the same point in time, which may not be relevant for diseases such as cancer which take many years to develop. However, these studies are relatively simple to conduct and suggest possible links for further investigation. Analytical epidemiology studies are more useful in hazard identification since they focus on groups of individuals and it is often possible to take into account factors that may contribute to a disease other than the exposure of interest (such as age and smoking).

## BOX 2A

### Hazard identification – the pesticide

#### Key observations

- Toxicity studies in experimental animals and models, including short term studies in rodent and non-rodent over a period of up to 10% of the expected lifespan (i.e. 90 days in rat, 1 year in dog)
- Maximum dose limit of 5 g test material per kg bodyweight
- Studies to use both male and female animals
- Test material to not exceed 1% of the total diet
- Adequate nutritional status to be maintained
- Additional studies may be required to investigate neurotoxicity
- Additional human data, including data on accidental poisonings, health effects observed in workers (manufacturing plants, spray operators, etc) may also assist the evaluation

#### Hazard identification

- Consider whether a threshold mechanism can be assumed
- Identify the effect seen at the lowest dose levels (the critical effect)
- Consider the relevance to humans

## BOX 2B

### Hazard identification – aflatoxins

#### Key observations

- Aflatoxins are potent mutagens, i.e. they produce permanent changes in the genetic material
- They have been shown to induce liver cancer in most animal species that have been studied
- Most epidemiological studies show a correlation between exposure to aflatoxin B1 and increased incidence of liver cancer
- Aflatoxins are metabolised in humans and test animal species to produce the same reactive intermediate, which is considered to be responsible for generating changes in the genetic material
- It is estimated that 50–100% of cases of liver cancer are associated with persistent infection with hepatitis B and / or hepatitis C

#### Hazard identification

- Aflatoxins are considered to cause liver cancer in humans, based upon the weight of evidence
- Uncertainty relates to the extent to which aflatoxins are able to induce liver cancer in the absence of hepatitis infection

Analytical epidemiology may take the form of case-control studies, or cohort studies, both of which have the capacity to provide quantitative information on exposure, and hence allow the dose-response relationship to be established. Case-control studies compare individuals with a disease (the "cases") and individuals without the disease (the "controls") in order to identify differences in exposure that may have contributed to development of the disease. They are of particular value in investigating possible causes of rare health effects. Cohort studies examine groups of individuals with and without a known exposure in order to establish whether the incidence of disease differs, and are therefore analogous to toxicity studies. They may be conducted retrospectively, using information on past exposure, or prospectively, following groups of individuals to see if they will develop the disease in the future. Prospective studies form the gold standard for epidemiology studies because they allow much greater control and accuracy in measurement of exposure and diagnosis of the disease. However, they need to be conducted over long periods of time in order to study the development of chronic diseases.

It is important to appreciate that epidemiological studies are usually observational and that observation of a correlation between a type of exposure and a health outcome is not evidence of a direct causal relationship. The observed association could be totally coincidental, or may be due to a second effect that has not been measured in the study, such as changes in lifestyle due to economic development. A recent development, known as molecular epidemiology, aims to provide stronger evidence for causal links. Biological samples (e.g. blood, urine) are obtained from the individuals in the study and analysed for markers of exposure and / or of effect in order to compile detailed information on the relationship. In some circumstances, it may be possible to conduct intervention studies, in which the exposure is changed in order to establish whether it results in changes in the disease, or in early markers of a chronic disease.

In 1965, Hill defined several criteria which provide evidence of a causal link between exposure and effect in epidemiology studies. These include:

- The strength of association – a large difference in the incidence of disease between exposed and unexposed populations, or in the occurrence of previous exposure between patients with the disease and controls without the disease.
- The consistency of the association – whether it is seen in different studies "conducted by different investigators in different places, circumstances and times".
- The temporal relationship – exposure must precede the disease, and where applicable, by a sufficient time to allow for development of chronic diseases such as cancer.
- The biological gradient of the association – risk of disease should increase with increased level of exposure.
- The specificity of the association – correlation with a disease that is rare in the absence of the exposure can provide strong evidence for causality, although absence of specificity does not exclude causality.
- Biological plausibility – supported by results of studies in experimental animals and understanding of the aetiology of the disease.

Case reports describe an effect in an individual, or in a small group of individuals, and tend to be anecdotal. Because they are highly subjective, and often lack statistical analysis, they are of very limited value in hazard identification.

Unlike epidemiological studies and case reports, human volunteer studies allow for control of exposure, which provides greater confidence in the link between exposure and effect. However, for ethical reasons they are limited to short term exposures that will result in, at most, mild temporary effects, and moreover they do not allow the evaluation of effects in high risk groups. They are probably of greatest value in studying the fate and distribution of chemicals in the human body compared with that in the experimental animals used for toxicity studies.

Clearly, it would not be ethical to give a chemical to human volunteers unless there is a reasonable degree of confidence that they will not be harmed. Needless to say that they are capable of giving informed consent. Furthermore, there is a lack of adequate epidemiological data for most chemical substances, and therefore it is necessary to conduct toxicity studies in laboratory animals.

### *Studies in laboratory animals*

A new synthetic chemical for which there is expected to be widespread human exposure in food and/or drinking water is normally subjected to an extensive battery of toxicity studies, as described in detail in the ILSI Europe Concise Monograph: *The Acceptable Daily Intake: A Tool for Ensuring Food Safety*. Several guidelines and quality assurance measures have been established to ensure that the results of toxicity studies are suitable for use in safety evaluation and that differences between treated animals and control animals can be attributed to the treatment of interest.

Guidelines for study design are defined by the Organisation for Economic Cooperation and Development (OECD) and by scientific committees and organisations responsible for regulation of different types of chemical in food or water. These guidelines are not strictly defined protocols and allow flexibility in many aspects, for which the methods selected should be justifiable in terms of the intended use of the chemical. The endpoints that are included in a particular study are selected taking into account the nature of the chemical under investigation and its likely usage and effects. Guidelines for testing methods are continually reviewed, in the light of new scientific understanding, to allow incorporation of new approaches that have been shown to be valid and relevant. Toxicity studies should be conducted in compliance with the principles of Good Laboratory Practice (GLP), which is a quality assurance scheme, initially introduced to prevent falsification of the results. It defines standards relating to management structures, training of personnel and laboratory maintenance within the organisation, and the conduct, recording and reporting of all aspects of a study. The principles of GLP are internationally agreed upon and compliance must be accredited by the appropriate national regulatory body.

The endpoints of a toxicity study are the observations and measurements of potential toxic effects, summarised in Table 1. Several observations such as changes in general appearance and behaviour and monitoring of food consumption and weight gain are made regularly throughout the duration of a toxicity study. At the end of a study, and at interim points during a long-term study, groups of animals are culled and autopsies performed. At autopsy, visible changes are recorded, and major organs are weighed. Samples of blood and various tissues are taken for biochemical assays and microscopic observation. Any animals found to be suffering during the course of a study are sacrificed and an autopsy is performed to attempt to establish the cause of the discomfort.

Toxicity endpoints may be categorised as functional or morphological changes. At the simplest level, functional changes include a slower rate of weight gain in test animals compared to control animals, which is unrelated to lower food intake. Reduced weight gain is not necessarily associated with detectable pathological

## TABLE 1

### Examples of types of adverse effect caused by chemical agents

| | |
|---|---|
| *Functional changes* | • Reduced weight gain |
| *Morphological changes (other than cancer)* | • Organ enlargement<br>• Histopathological lesions |
| *Mutagenicity* | • Heritable changes in DNA, genes and chromosomes, with the potential to cause cancer or fetal abnormalities |
| *Carcinogenicity* | • Cancer |
| *Immunotoxicity* | • Sensitisation (leading to hypersensitivity or allergy)<br>• Depression of the immune system (leading to increased susceptibility to infection) |
| *Neurotoxicity* | • Behavioural changes, deafness, tinnitis, etc. |
| *Reproductive effects* | • Impaired fertility<br>• Embryotoxicity (spontaneous abortion)<br>• Teratogenicity (fetal deformities)<br>• Other developmental effects |

effects but may be the only change seen with relatively innocuous chemicals. Functional changes may also include changes in enzyme activity. If tissue abnormalities, such as changes in organ weights, are observed, it is necessary to consider whether these have occurred as a direct result of the chemical under investigation or may have arisen indirectly, for example, due to interference with the animal's nutritional status. Expert committees review all the reported effects to establish whether they can be considered:

- directly attributed to the chemical;
- relevant to man;
- "adverse."

Three to five different dose levels may be used and, ideally, these should be selected to produce a range of responses, with only the lowest dose having no observable adverse effect (this dose would then be referred to as the no-observed adverse effect level [NOAEL], which is an important concept in CRA). Preferably, the increment of the doses should not exceed 5, and in exceptional cases 10.

### *Alternative techniques*

Ethical considerations demand that we should minimise the use and suffering of laboratory animals. As a result, there is considerable interest in development of alternatives to animals, such as computer modelling and *in vitro* approaches. These models often provide useful mechanistic information, but at the current time they are not considered acceptable as complete replacements for *in vivo* testing. They are, however, a very useful additional tool and should be combined with animal experimentation.

The structure and properties of a substance may provide clues for the types of toxicity to be expected.

Structure-activity-relationships (SAR) are based either on expert knowledge of similar chemicals or, for some specific types of effect, on computerised databases that highlight particular chemical groups of concern. Such analyses are not sufficiently reliable to preclude the need for toxicity testing, but can be used to indicate the types of toxicity studies that are of greatest importance. Another recent development is likely to be particularly useful with respect to risk assessment of chemical substances found at low concentrations in food and water. Review of the potential toxic effects of several substances has indicated that it is possible to establish a Threshold of Toxicological Concern (TTC), based on the concept that for any chemical substance there must be a level of intake below which there is no significant risk to health. Substances present in food or water at such low levels that the potential daily intake would be below this threshold could be assumed to pose no appreciable risk to health. Substances present in higher amounts, such that the threshold could be exceeded, would then be prioritised for full risk assessment.

### *Critical effect*

Review of all the available data may indicate that one or more different types of adverse effect are associated with the chemical under investigation, perhaps depending on the animal species, dose levels and dosing periods used in the different studies. The major sources of uncertainty relate to the relevance and adequacy of the endpoints measured in the studies. In general, the effect that is seen at the lowest dose level in long-term animal studies is considered to be of most importance, provided it is judged to be relevant to humans. This is referred to as the critical effect and is used as the primary basis for risk assessment. (Under current accepted risk assessment procedures, the severity of the effects is a secondary consideration). However, it is also important to consider the relative sensitivity of humans compared to the animals used in the study.

## Microbiological hazard identification

Microbiological hazard identification focuses on the agent, the likelihood of its association with food or water and consequences of its presence, as defined by CODEX: "*identification of biological, chemical and physical agents capable of causing adverse health effects*". It aims to identify the likelihood of the presence of known pathogenic microorganisms or microbial toxins, and to evaluate whether they have the potential to cause harm when present in food or water. This is based primarily upon information in the scientific literature, and from public health and surveillance data compiled by the food and water industries and government agencies relating to the amounts, frequencies and sources of the microorganism (Box 2C). Pathogenic microorganisms in food or water cause diseases that, in the early stages, may be reflected by symptoms such as nausea, influenza-like symptoms, gastroenteritis and diarrhoea. Affected individuals may recover, or may subsequently develop focused systemic diseases or wide-ranging conditions, such as reactive arthritis, Guillaine-Barré syndrome, etc.

For many established food- and water-borne pathogens, much information is already available and there is little formal requirement for additional testing of potential adverse effects, as is required for chemical substances. This is more analogous to the evaluation of aflatoxin shown in Box 2B, for which there is a steadily accumulating body of information in the scientific literature, than for a new chemical substance that is subjected to a defined testing strategy.

However, there is a need for more information on newly emerging pathogens and this may be obtained using similar approaches to those for chemical hazards (i.e. clinical studies, epidemiological and animal studies). In addition, specific investigations are needed on the ecology, physiology, growth characteristics, detection

## BOX 2C

### *Hazard identification* – Listeria monocytogenes

**Key observations**

- It causes listeriosis in humans, with symptoms including mild diarrhoea, meningitis, septicaemia, abortion and stillbirth
- Epidemiological evidence suggests that most exposure is foodborne
- Cases are infrequent but 20 to 40% are fatal in susceptible individuals
- Illness is associated with only a few virulent strains
- Major risk factors include immunosuppression, pregnancy and age

**Hazard identification**

- Milk and dairy products, particularly soft cheeses are implicated in outbreaks of listeriosis
- Cheeses have been the vehicles for four major outbreaks, one in the USA, one in Switzerland and two in France

methods and identification of species and strains of microorganism associated with the food in question. Because of the potential for identifying an infective agent, and because overt disease frequently occurs relatively quickly after exposure to the pathogen, epidemiological studies are more likely to give evidence of causality than is the case for chemicals that cause chronic diseases.

## *Hazard characterisation*

Whereas in hazard identification, the emphasis differs for microbiological and chemical agents, the processes of CRA and MRA are much more closely aligned for the hazard characterisation stage and a common definition can be described: "*qualitative and/or quantitative evaluation of the nature of the adverse health effects associated with biological, chemical and physical agents. For chemical agents, a dose-response assessment should be performed. For biological or physical agents, a dose-response assessment should be performed if the data are obtainable*". The dose response assessment is defined as "*determination of the relationship between the magnitude of exposure (dose) to a chemical, biological or physical agent and the severity and/or frequency of associated adverse health effects (response)*". In microbiology, a dose response is usually less detailed and often only records the incidence of a particular effect (illness) against dose of the agent. The overall aim for both is to estimate the nature, severity and duration of the adverse effects resulting from ingestion of the agent in question. In both cases, it is advantageous to consider two major issues:

1. the fate and distribution of the chemical or microbiological agent in the body; and
2. the action of the chemical or microbiological agent on tissues or functions of the body (often referred to as the host in studies of microbiological agents).

The first of these issues could be considered more complex for microorganisms than for chemicals, because of their capacity to replicate, and consequently it is currently often not evaluated. Hazard characterisation is related to exposure assessment for microbiological agents, since both processes focus on the capacity for replication (in the human host and in the food chain, respectively). In contrast, for chemicals, hazard characterisation and exposure assessment are

## BOX 3A

### Hazard characterisation – the pesticide

**Toxicity studies in experimental animals**

- Establish the highest dose level without effect (the NOAEL) in the study that identifies the critical effect in Box 2A, or model the dose response relationship to identify a benchmark dose

**Toxicokinetic studies**

- Data on absorption, distribution and excretion, and identification of major metabolites
- Investigation of the effects of dose level and duration on the metabolism of the test material
- Comparative studies in human volunteers (or using human tissues) to support extrapolation of animal toxicity data to humans

**Biochemical studies**

- Studies of the mechanisms of effect of the pesticide, taking into account effect on target species (pest) as well as on mammalian systems

**Hazard characterisation**

- Consider whether toxicokinetic data and current understanding of the toxicological mechanism indicate that humans are similar, less or greater in sensitivity to the critical effect, compared with the test species
- Consider whether to use a default safety (uncertainty*) factor (100) or if data support selection of a lower factor, or level of uncertainty warrants selection of higher factor
- Establish acceptable daily intake (ADI), where:

$$ADI = \frac{NOAEL}{safety\ factor}$$

* In recent years, there has been a trend towards the use of the term “uncertainty factor”, see Glossary.

completely separate processes, which are brought together in the risk assessment.

### Chemical hazard characterisation

For chemical agents, hazard characterisation is closely linked to hazard identification. It often is based on evaluation of the same studies, particularly laboratory animal toxicity studies which identify the critical effect (Box 3A) and also on epidemiological studies if they include adequate exposure data (Box 3B). Hazard identification revealed the type(s) of toxicity associated with a particular substance that were considered to be of

## BOX 3B

### Hazard characterisation – aflatoxins

- Epidemiological data on the dose-response relationship are influenced by concurrent infection with hepatitis B
- Data indicate two separate potencies for aflatoxins – in populations with common hepatitis infection and in populations in which chronic hepatitis infection is rare
- The shape of the dose-response curve is unknown, which increases uncertainty related to application of mathematical models for extrapolation from animals to man, and from high dose to low dose

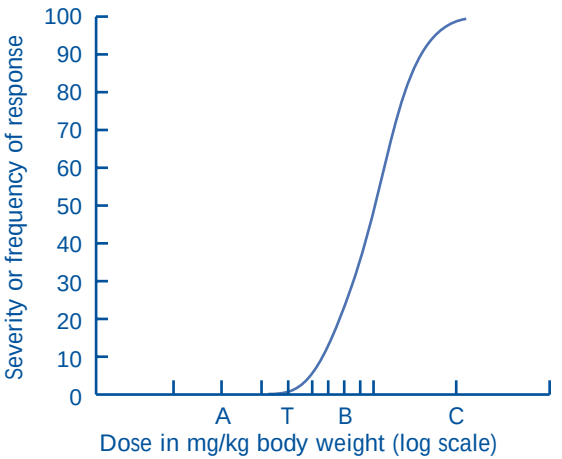
relevance to humans. This could be viewed as a qualitative species extrapolation, exerting knowledge of toxicology to reach an opinion. The focus in hazard characterisation is on the relationship between dose and response that is revealed in these studies and subsequent estimation of dose levels that may cause that response in humans, i.e. a quantitative species extrapolation.

### *Dose-response relationship for threshold effects*

A typical dose response curve is shown in Figure 2. A similar curve is produced whether considering the frequency of an all-or-nothing response, such as death, or a continuously variable response, such as the severity of effect. No toxicity is seen at the lowest doses. As the dose is increased, a small number of individuals may be affected, representing the most vulnerable in the group. As the dose is increased, the majority, and eventually, all of the exposed population will be affected. Alternatively, for a variable response, the severity of response increases as the dose increases.

**FIGURE 2**

**Typical dose-response relationship**




Points A,B and C are dose levels used in the toxicity study
Point T is the theoretical threshold dose

Based on our current understanding of the mechanisms of toxicity, we assume that there is a "threshold" for most types of toxic effect. A threshold is a level of intake below which no effect is produced, because the body's natural defence mechanisms will reverse any minor changes caused and thus maintain homeostasis. Point T represents the threshold between dose levels with and without effect. As it is not possible to identify an absolute value for the threshold, it is normal to use an alternative experimentally derived dose value as a surrogate for the threshold. The most common approach is to use the NOAEL, the highest dose level without adverse effect in the toxicity study that demonstrated the relevant critical effect(s).

The NOAEL is not an inherent property of a substance; it is an experimental observation, the value of which is dependent on the way in which a toxicity study is designed (as discussed in the ILSI Europe Concise

Monograph: *Acceptable Daily Intake*). In most cases, the observed NOAEL will be lower than the threshold, e.g. point A on Figure 2, with points B and C representing the middle and high-dose levels used in the toxicity study.

If effects were seen at all dose levels used in the key toxicity study, then it is not possible to identify a NOAEL, and the lowest-dose-level used is referred to as the lowest-observed-adverse-effect level (LOAEL). Alternative approaches may be based on mathematical modelling of the dose response curve to determine a dose level associated with a low incidence (e.g. 10%) of effect, which is referred to as a benchmark dose.

### *Inter- and intraspecies differences*

Modern toxicity studies are conducted in laboratory animals (mostly rodents), which have been bred specifically for the purpose. The animals are of defined genetic strain, free of specified pathogens and maintained under strict conditions. The environmental and genetic controls mean that the individual animals used in a toxicity test are very similar to each other and therefore respond to toxic insult in a relatively homogeneous manner. Animals are treated with relatively high doses of chemical to obtain an observable response.

When considering a risk assessment for human exposure via food or drinking water, we need to focus on prolonged (potentially life-long) exposure of entire human populations to relatively small doses of chemical. There is wide variability within the human population and susceptibility to toxicity may be influenced by many factors. Some are internal to the individual, including genetic differences, gender, age, hormonal status and disease status. Others are external factors and include components of the diet and substances that we are exposed to from the environment, the influence of which may vary considerably at different times for one individual.

Therefore, when attempting to use information from animal tests to predict risk to people, we need to be aware of two major categories of variability:

1. The differences between the animal species used in the toxicity study and human beings in general, i.e. interspecies variability;
2. The variability in sensitivity that might be expected amongst the entire human population, i.e. inter-individual variability (intraspecies variability).

As already noted, hazard characterisation needs to consider the fate of the agent and the effects of the agent on the host as related to the dose. For a chemical, the fate primarily relates to what happens to it within the body (i.e. from the point of ingestion), as summarised in Figure 3, and how this changes with the dose.

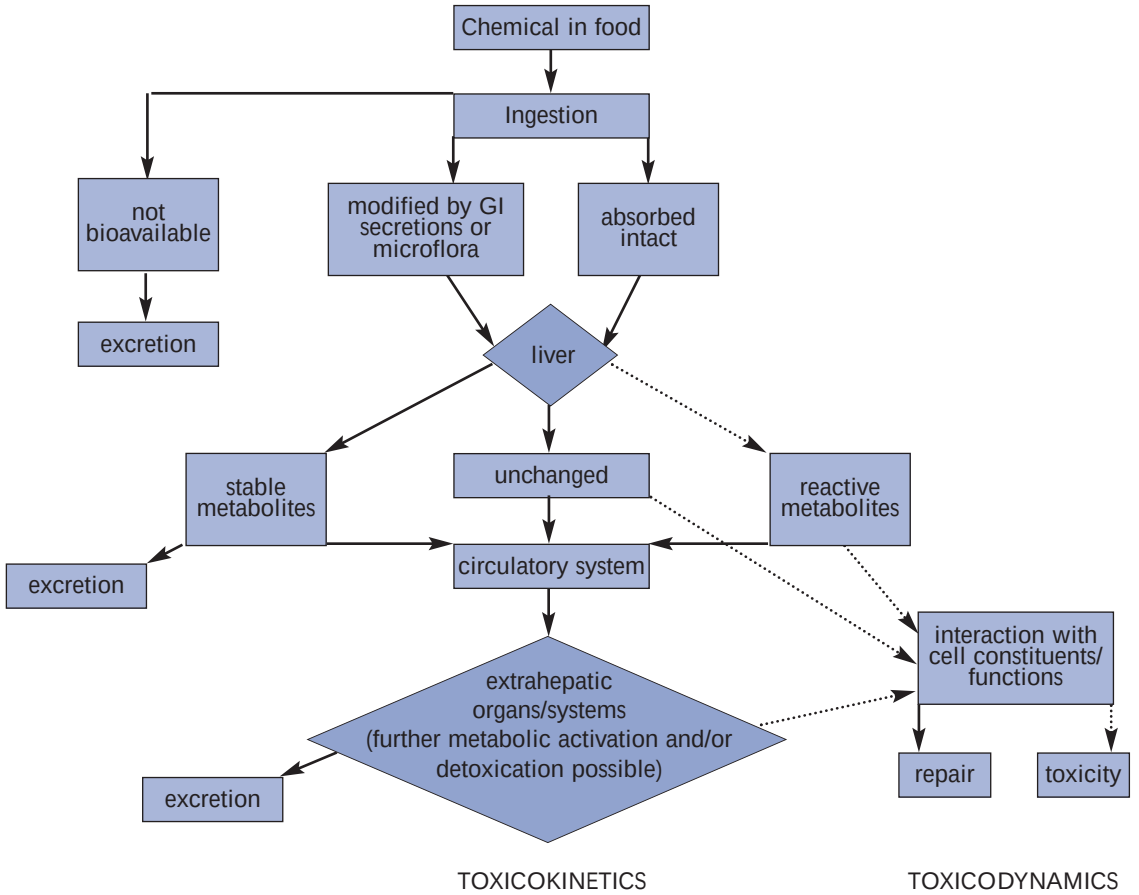
The fate of a chemical in the body relates to the processes of absorption into the body (via the gastrointestinal tract for ingested substances), distribution around the body, modification to different chemical structures (known as metabolism or biotransformation) and elimination or excretion from the body. These processes are referred to as *toxicokinetics*.

The effects of the chemical on the body relate to interactions of the chemical with the body, e.g. interactions with systems, tissues or cells, and with the potential to initiate a toxic response, and possible repair and regeneration of the affected tissue or system. These processes are referred to as *toxicodynamics*.

Together, the toxicokinetics and toxicodynamics are the major sources of differences between animals and humans and between different individuals. Scientific understanding of toxicokinetics and toxicodynamics is increasing rapidly, but detailed knowledge is limited to very few chemical substances and is not comprehensive even for these. A third aspect of consideration is the

**FIGURE 3**

**The fate of an ingested chemical in the body, possibly leading to adverse effects**




Dotted arrows indicate pathways leading to adverse effects

difference in size between the animal species used in toxicity studies and humans. It is normal procedure to correct for size differences by normalising exposure in relation to bodyweight, e.g. expressing dose as milligram substance ingested per kilogram of bodyweight, and assuming an average human weight of 60 or 70 kg as appropriate.

As shown in Figure 3, the overall biological response results from the balance of a number of different reactions. Comparative information on the balance of these reactions in animals and humans can be used to aid prediction of potential effects in humans from results of animal studies. In addition, knowledge of the range of variation between individuals will further

reduce uncertainty in establishing safe levels for the entire human population. In practice, the available data are generally incomplete, and such information is normally used in a qualitative rather than a quantitative manner.

### *Application of safety factors to the NOAEL*

Derivation of an acceptable or tolerable human dose from the NOAEL, LOAEL or benchmark dose is achieved by allowing a margin of safety, or uncertainty factor, in recognition of the uncertainty involved in extrapolating to humans. A value of 100 is frequently used as the safety or uncertainty factor, which consists of a factor of 10 to allow for interspecies differences, and a factor of 10 to allow for interindividual differences in the human population. Thus the tolerable human dose would be the NOAEL derived from the critical effect in the most sensitive species divided by 100. The uncertainty factor of 100 did not initially have a scientific basis, but recent analyses have shown that it has validity as a worst case approach. Lower factors may be used for specific healthy populations, or if human data are available that reduce the uncertainty in species extrapolation. Moreover, the uncertainty factors used for macro- and micronutrients are often much lower than those used for food additives or contaminants. If the safety of some vitamins and essential elements was evaluated according to the stringent procedures used for additives and contaminants, the resulting acceptable daily intake would be lower than the recommended daily allowance (RDA) for optimum nutrition.

If specific data are available relating to the differences between humans and animals, then "data-derived uncertainty factors" may be used in preference to the default values. Alternatively, higher factors may be used to allow for additional uncertainty if important information is missing from the toxicological database such as:

- absence of long-term studies;
- absence of particular studies, such as on reproductive effects;
- use of LOAEL instead of the NOAEL.

Substances that are considered acceptable for use as pesticides are likely to have some toxic effects, but these should be considered to be threshold-related so that an acceptable intake can be established (as in Box 3A).

### *Non-threshold effects*

There is a small number of effects which may theoretically result from damage by a single molecule to a single cell and a precautionary approach does not allow us to assume a threshold, even though control processes and repair mechanisms may be effective at low levels of exposure. The potential to cause cancer as a result of damage to the DNA is the main example of a non-threshold effect. The accepted regulatory procedure is to assume that there is no safe level for substances that cause such effects and it then becomes necessary to attempt to evaluate the risk associated with different levels of exposure. Ideally, this would be based upon results of epidemiological studies providing information on the dose-response relationship. In practice, there are very few substances for which quantitative risk assessment can be based upon epidemiology, but aflatoxin provides an example of where this has been attempted (Box 3B).

Various mathematical models have been developed to extrapolate the dose-response curve from the high doses used in the animal carcinogenicity studies to the low-dose range relevant to human exposure. Linear extrapolation has been widely used, particularly in the United States, if there is evidence of a linear dose-response, or as a policy default if there is no evidence of either linearity or non-linearity. This approach is not uniformly accepted by regulatory authorities as there are considerable uncertainties associated with it.

Another approach involves estimation of the dose associated with a small incidence (e.g. 5%) of tumours in the animal studies (benchmark dose) or from epidemiological studies on human populations. This dose may be divided by a large margin of protection (e.g. 1 million) to derive an exposure level at which the risk is defined as acceptable. Some authorities may refer to this as a "virtually safe dose". For unavoidable contaminants, it may not be possible to always ensure that contamination is confined to a level that is considered to be virtually safe. In this situation, some authorities (as risk managers) may stipulate that the level should be minimised as much as possible in each circumstance in which the contaminant arises. This may be referred to by the terms "as low as reasonably practicable" (ALARP), or "as low as reasonably achievable" (ALARA).

More recently developed models allow incorporation of data on the mechanisms of cancer development and toxicokinetic differences between animal species and humans, although, so far, there are very few examples for which sufficient data are available to support this type of evaluation.

## Microbiological hazard characterisation

The aim is to provide an estimate of the nature, severity and duration of the adverse effects that may result from ingestion of harmful microbiological agents present in food or water (Box 3C). Quantitative estimates should be made if data can be obtained on the dose-response or attack rates (how many individuals are ill after ingestion). As with chemical hazards the major factors for consideration relate to those governing the fate of the microorganism and the effect on the human host. The factors may relate to:

A: Characteristics of the microorganism.

B: Interaction of the microorganism with the food matrix and the host.

C: Dynamics of infection and / or intoxication.

D: The host, and the host's health status and predisposing factors.

Of these, the first three categories are specific to microbiological agents, whereas the fourth is directly comparable to the chemical hazard characterisation.

### *A: Characteristics of the microorganism*

In theory, basic information is required on the nature of the factors that allow the organism to survive the passage through the stomach, resist the chemical, physical and immunological defences, establish a niche in the intestinal tract, and multiply, but this information is currently lacking. The virulence of the microorganism is determined by many factors, such as its acid resistance, the capability to bind to epithelial cells, penetrate cells and / or to produce toxins.

Information is also required on the ease of transmissibility of the microorganism between individuals, which influences the size of potential outbreaks of disease beyond considerations of intake. It should be noted that the properties of the microorganism may tend to change

**BOX 3C**

### *Hazard characterisation –* L. monocytogenes

- Dose-response models used to estimate the probability of illness resulting from a single serving for the 20% of the population considered to be at high risk (pregnant, elderly or taking immunosuppressive drugs).

with time, whether due to natural mutations or to interactions with its environment (food, water), leading to altered pathogenicity and virulence.

### *B: Interaction of the microorganism with the food matrix and the host*

A variety of factors in food may influence the amount of a microorganism needed to cause infection or disease, including fat content, iron content, buffering of pH, temperature, background microflora, presence of preservatives, whether the food is liquid or solid and the circumstances of ingestion. Many of these may act by influencing the physiological state or survival of the microorganism. For example, enhanced survival could be associated with:

- increased stomach pH, due to age or use of antacids and / or intake of food with a strong buffering capacity;
- decreased residence time in the stomach (e.g. rapid transit of liquids through an empty stomach);
- protection of the microorganisms from stomach acid by entrapment in lipid droplets or in highly buffered foods.

### *C: Dynamics of infection*

The frequency and severity of adverse effects produced by a pathogen are influenced by the amount ingested in a manner analogous to that observed with chemical agents. Thus, increasing levels of a pathogen in food or water at the time of consumption will generally result in a greater proportion of the population becoming ill, a decrease in incubation period (the time taken to onset of disease following exposure), and possibly in the severity of disease in individuals. However, analysis of different studies may be complicated by the fact that the term infection was ill-defined, i.e. whether it pertained to colonisation only or to overt symptoms of disease.

### *D: Host susceptibility*

As with chemical susceptibility, there may be marked interindividual differences in susceptibility to microbiological agents. For example, decreased resistance to infection may be associated with:

- genetic factors that compromise the immune response or predispose to complications, e.g. reactive arthritis;
- reduced immune status of vulnerable subpopulations such as premature infants, the elderly, pregnant women, individuals taking immunosuppressive drugs;
- concomitant infections, reduced liver and / or kidney function;
- nutritional deficiencies, stress.

Conversely, previously exposed individuals may have developed immunity and therefore be less susceptible to re-infection.

Which of these factors are important will depend on the type of microbiological hazard. Susceptibility to infectious pathogens is more likely to be affected than susceptibility to toxins. The risk assessment should indicate which sub-population is the subject of the response. If an overall population assessment is done, then the susceptibility of vulnerable sectors should be used as a benchmark.

## Dose-response relationship

For both chemical and microbiological agents, the dose-response assessment involves the estimation of whether harmful effects are likely to arise at different levels of human exposure or intake. For practical purposes, it is generally assumed that a single cell of a pathogen could result in infection, in the same way that it is assumed that a single molecule of a DNA-damaging chemical could result in cancer. If a microorganism acts by production of

a toxin, it is likely that a threshold level of toxin will be required in order for adverse effects to occur (as in Figure 2). However, because pathogens proliferate in the body, it is not possible to associate a level of pathogen intake with production of a threshold level of toxin. Therefore, the assumption of no threshold is considered to be a suitably cautious approach in microbiological risk assessment. However, there may be a very low probability that a single microorganism would cause a foodborne disease. One model used to determine a dose-response curve for *Listeria monocytogenes* estimated that this probability would be one in $10^{14}$.

For microbiological agents, the aim may be to obtain quantitative information on the probability of human illness resulting from exposure to a certain level of the agent (as in the example in Box 3C). Such information may be obtained from several sources such as:

- foodborne disease analysis;
- food surveillance data;
- population characteristic surveys;
- animal trials;
- human volunteer studies.

Several mathematical models have been developed to describe the dose-response relationship for pathogen infection, often incorporating a measure of the variability within a population (i.e. the effect of vulnerable populations) rather than assuming an overall average probability of risk. For *L. monocytogenes*, separate models have to be produced for the normal (low risk) population and for high-risk populations, including pregnant women, immunocompromised individuals and the elderly.

Bacterial toxins may be formed in a food, and when the dose is high enough, they may provoke various diseases. Risk assessment of such toxins follow the same protocols as those described for chemicals.

## Exposure assessment

Exposure assessment is the "*qualitative and/or quantitative evaluation of the likely intake of biological, chemical or physical agent via all relevant sources*". For food, the level ingested will be determined by the changes in the levels of the agent along the supply chain and after storage and use in the relevant food. The effect of the final levels needs to be related to different individuals and populations. Exposure is normally expressed in different terms for chemicals and microbiological agents, and this relates to the different ways in which exposure may lead to harmful effects. For a chemical, extremely high levels of contamination would generally be required to produce an acute adverse response from a single ingestion. This could possibly be envisaged following a major chemical accident resulting in contamination of drinking water, but is not common for chemical contaminants in food. Prolonged intake of a chemical agent at relatively lower levels may lead to accumulation within the body, eventually leading to chronic toxicity. Thus, the exposure unit of interest is the amount taken repeatedly, potentially over the entire lifespan. In contrast, a single exposure to a pathogenic organism has the potential to result in infectious disease. Thus, exposure to chemicals is expressed in terms of daily or weekly intake over a lifetime, whereas exposure to microbiological agents usually relates to a single serving of contaminated food. However, microbiological exposure assessment may need to trace the potential for contamination to occur at all stages of food production from farm to fork. If there is a good "killing" step in the process, then it may be sufficient to consider contamination after this step.

### Chemical exposure assessment

For a chemical substance, exposure may relate to external contact or it may relate to intake into the body. In the context of risk assessment for food and drinking water, the primary source of exposure is via ingestion.

The degree of exposure is determined by the amounts of food and water consumed that contain the chemical, and the levels of chemical contained in those foods (Boxes 4A and 4B). An estimation of intake is based upon a number of assumptions and subject to considerable uncertainty and variability. Clearly there are enormous differences in dietary habits between individuals, and even for a single individual consumption of specific food items will vary from day to day, and with different seasons and periods in life.

Information on patterns of food consumption are obtained from surveys of the type and quantities of food and drink consumed by individuals over specific periods, or from food frequency questionnaires on how often an individual consumes various types of food. It is then necessary to estimate the amounts of food that are consumed per portion, which can be supported by information from diet diaries, in which subjects record everything they eat over a period of time. For contaminants of specific concern, monitoring programmes may be established with regular analysis of batches of foods in different regions, such as those for aflatoxin (Box 4B).

The more sophisticated methods may allow mathematical analysis of the range of intakes amongst consumers. Risk assessments are based upon an intake towards the upper extreme of the range in order to ensure that the majority of consumers are protected from possible adverse effects. It is worth noting that different agencies use different figures to represent extreme consumers, and this may lead to differences in interpretation of results. For example, whereas the European Union uses the 95th percentile (i.e. 95% of the population consumes at this level or less), the United States uses the 90th percentile. Developments in statistical techniques allow the variability and uncertainty in exposure data to be expressed in terms of a probability distribution. As new information is

## BOX 4A

### *Exposure assessment – the pesticide*

**Estimation of maximum daily intake from foods**

- Content in foods based on Maximum Residue Level (MRL), determined in studies of pesticide application to various crops, using principles of best agricultural practice
- High level consumption of different contaminated food commodities estimated from survey data
- Calculation of maximum daily intake (worst case)

**Estimation of potential daily intake from foods**

- Content in range of foods based on chemical analysis
- Consumption of different food commodities estimated from dietary records
- Calculation of potential daily intake (more realistic)

**Pesticide residues in drinking water**

- Based on worst case maximum of drinking water quality standards where available
- Analytical data on surface water in vicinity of pesticide trials
- Assume 2 litres consumed per day for adults

**Calculation of total intake from food and/or drinking water as appropriate**

- Maximum or potential intake, depending on nature of the available data and the assumptions made

## BOX 4B

### Exposure assessment – aflatoxins

**Sources of aflatoxin**

- Most commonly associated with groundnuts, dried fruit, tree nuts, spices, figs, crude vegetable oils, cocoa beans, maize, rice, cottonseed and copra
- Levels of contamination dependent on temperature, humidity, soil and storage conditions

**Aflatoxin content in contaminated crops**

- Analytical data from monitoring of different foods in different regions
- The data are not considered to represent accurately the full range of contamination

**Amount of imported crops consumed**

- Based upon established standard regional diets
- Consideration of effects of food processing on actual intake

**Estimation of intake**

- Based upon reported range of contamination and amounts consumed

**Investigation of alternative scenarios**

- Estimation of the impact on total intake that would be achieved by successfully restricting maximum levels of contamination to 20 or 10 µg / kg of foodstuff, e.g. for aflatoxin in maize in the USA:

| max level (µg/kg) | % crop rejected | mean level (µg/kg) |
|---|---|---|
| - | 0 | 4.7 |
| 20 | 3.9% | 0.9 |
| 10 | 6.2% | 0.6 |

received, it can be used to refine and improve the description, providing a more realistic assessment of the range of risk in given situations.

Assessing the intake of chemicals from drinking water is more straightforward than for chemicals in food. It is commonly assumed that the average adult drinks 2 litres of water per day, including hot drinks such as tea and coffee, and water in meals. The total volume is likely to include tap water, bottled water and commercially prepared beverages, but as a worst-case scenario, it can be assumed that it is all a potential

source of intake. Consideration must again be given to specific populations who may be at greater risk because of extreme dietary patterns – particularly infants fed entirely on formula-milk made up with water.

Not all of a chemical contained in a foodstuff is available to be absorbed into the body. Some may be trapped within the food matrix, depending on the nature of the food in which it is contained as well as the nature of the chemical itself. The degree of absorption in the stomach or intestines is also influenced by the nature of the chemical, e.g. its solubility. Thus, the actual amount absorbed into the body (referred to as the internal dose) may be a small fraction of the amount ingested. For a few chemicals, analytical methods (commonly referred to as biomarkers) are available that allow quantification of the internal dose, or of the dose found at the site of toxicity. However, in the absence of specific information, it must be assumed that 100% of the ingested chemical is absorbed into the body.

Intake of a chemical is normally expressed as the amount ingested per unit time (e.g. mg/day) and related to the body weight of an average individual (i.e. mg/kg bodyweight per day). This allows ready comparison of intakes in human populations with the doses used in animal toxicity studies.

In some circumstances, exposure to a chemical may derive from many different sources, with the result that estimation of consumption and intake do not adequately reflect the potential exposure. Mathematical modelling approaches may then be used to determine the worst-case exposure scenario. An example of this is seen with emissions of dioxins from incinerators. Data on levels of dioxins within the incinerator chimney are used to derive levels of dioxin contamination of ground water, crops and livestock within the vicinity. The potential intake of total dioxins from all possible sources, including background pollution, is then estimated to determine the acceptability of the incinerator emissions.

## Microbiological exposure assessment

The goal of microbiological exposure assessment is to describe the level of viable microorganisms or microbial toxins in the food or drinking water at the time of consumption. It may indicate the actual or anticipated human exposure. For foodborne microbiological agents it may be based on the extent of food contamination, its use and consumption patterns, and on dietary habits, such as that used in chemical exposure assessment (Box 4C). For chemical agents, "the extent of food contamination" relates to the level of chemical that may occur in different types of food at the moment of ingestion. This is the same for foodborne microbiological agents, where exposure assessment includes an estimation of the frequency of contamination and a distribution of the concentration of the pathogen or toxin in contaminated products as well as the frequency of ingestion of the various concentrations based on dietary habits, preparation and/or use conditions as applied by ultimate consumers.

Assessment of microbiological exposure needs to consider several factors, including:

- the characteristics of the pathogenic agent (e.g. its resistance to heat, its growth range);
- the realistic association of the microbiological agent with a particular foodstuff;
- the treatments that a food undergoes from farm to fork, i.e. during production, processing, handling, distribution and preparation for the end-user, and the effects of these treatments on the level, physiological state and virulence of the microbiological agent;
- the type of treatment that the food undergoes and the level of control associated with it;

## BOX 4C

### *Exposure assessment* – L. monocytogenes

**Content in raw milk**

- 10% of herds infected with *L. monocytogenes* mastitis
- Contamination results from environmental sources in 0 to 8% of farms
- Estimation of growth during storage at the farm, transport to the cheese manufacturer and storage of the milk by the manufacturer
- Probability of milk being contaminated was estimated to be 67%
- Calculation of final concentration in raw milk

**Cheese processing**

- Estimation of number of *L. monocytogenes* in one cheese vat of 1000 litres
- Estimation of number of *L. monocytogenes* in one 250g cheese prepared from 2.2 litres of milk

**Estimation of intake**

- Single serving is assumed to be one-eighth of a 250g cheese, i.e. 31g
- Based upon data on sales of soft cheese, an average of 50 servings per person per year is estimated
- Probability of consuming contaminated cheese estimated to be 65%

**Investigation of alternative scenarios**

- Decreased contamination of cheese when cows with *L. monocytogenes* are excluded

- the potential for cross-contamination with pathogens from other foodstuffs during food processing, storage, distribution and use;
- any preservation factors, e.g. the conditions of temperature, pH, humidity, atmospheric gases, etc. in and around a foodstuff during packaging, storage and distribution;
- the nature of the foodstuff itself and whether it allows or inhibits growth or survival of the microorganism under different conditions;
- consumer use (i.e. handling and preparation).

The variability in the above factors also needs to be considered, and in particular the effects of scenarios representing best, most likely and worst conditions, such as improper storage. Consideration of microbial contamination of drinking water may be simpler than that for food, since there is less potential for growth.

The purpose of the exposure assessment is to estimate the real frequency of ingestion of the various concentrations of the agent. Different methods for exposure estimation include growth models, challenge tests, storage tests and surveys. When the hazard characterisation (dose-response) curve has been established, this real exposure can be used to estimate the resulting effect from the curve. Exposure assessments can be done through actual surveys of the product under study, or by modelling the occurrence and fate of the agent from “farm to fork” or just a few relevant stages in the food chain. Laboratory studies may provide information on behaviour of the microorganisms in different foods and under different storage conditions. Various mathematical modelling techniques have been developed to predict the growth, death or survival of microorganisms under different environmental conditions and hence the likely level of contamination at the time of consumption. These can be

combined with models of the dose-response relationship in order to model the risk of illness following a single serving of food, as in the example in Box 4C. Models are also available that estimate the uncertainty associated with the exposure assessment.

The final stage of microbiological exposure assessment is more comparable to that for chemical agents. Having estimated the level of contamination for the specified agent at the time of consumption, information is required on dietary habits of different populations in order to determine the numbers of individuals that could be exposed. Particular emphasis may be placed on groups at greater risk (such as pregnant women for *Listeria*). However, unlike the situation with chemicals, the focus is on the potential for exposure, and the level, frequency and concentration of exposure, and there will need to be additional consideration of food preparation and hygiene practices.

## Risk characterisation

Risk characterisation is "*the qualitative and/or quantitative estimation, including attendant uncertainties, of the probability of occurrence and severity of known or potential adverse health effects in a given population based on hazard identification, hazard characterisation and exposure assessment*". In simple terms, this means estimating how likely it is that harm will be done and how severe the effects will be. The outcome may be referred to as a risk estimate, or the probability of harm at given or expected exposure levels. Essentially, it is the same process for chemical or microbiological risk. It brings together the information on exposure and health hazards defined in the earlier stages of risk assessment, and outlines the sources of uncertainty in the data on which they are based. It summarises the estimates of potential risk in different ways, for example, different scenarios, and should identify the strengths and weaknesses of the estimates. If the data are adequate to support numerical quantification of the risk, then this is also included. The risk characterisation provides the primary basis for making decisions on how to manage the risk in different situations, i.e. whether it is necessary or feasible to reduce exposure, provide advice to susceptible subgroups, etc. The information provided will relate directly to the stated purpose of the risk assessment, as shown in Boxes 5A-C.

For chemicals, the results of risk characterisation may be comparison of estimated intake or exposure with the acceptable or tolerable level of intake, which is considered not likely to cause harm if repeated daily over an entire lifetime (Box 5A). Alternatively, it may be an estimate of the increased incidence of disease or death that would be associated with different levels exposure repeated daily over an entire lifetime (Box 5B). For pathogenic microorganisms, identifying a safe or acceptable level is more difficult because it depends on many variables, as already described, and whether the hazardous agent is an infectious pathogen or a toxin-producer, or both. The risk could be expressed in terms of probability of disease or death (Box 5C) such as:

- average probability of illness for adults from a single meal;
- average probability of illness for children from a single meal;
- change in probability of illness in children if processing or a raw material are changed;
- likelihood of disease per 100,000 individuals per year.

Because the risk managers may not be technical experts, the risk characterisation must be expressed in plain language and be clear, transparent and reasonable including uncertainties and assumptions. The risk manager will wish to understand the level of risk in order to make decisions on if and how it should be

## BOX 5A

### Risk characterisation – the pesticide

**Compare the total maximum or potential daily intake from Box 4A with the ADI from Box 3B**

- Intake lower than the ADI – proposed pesticide use acceptable
- Intake potentially higher than the ADI – consider risk management options

## BOX 5B

### Risk characterisation – aflatoxins

**Review of potency estimates based upon epidemiological evidence**

- *In absence of hepatitis infection*. For every ng aflatoxin consumed per kg bodyweight per day, there will be an estimated additional 0.1 cancers / year / million people
- *In presence of hepatitis infection*. For every ng aflatoxin consumed per kg bodyweight per day, there will be an estimated additional 3 cancers / year / million people

**Potential impact of applying hypothetical standards for permissible levels of aflatoxin in food**

- In areas with low levels of hepatitis infection and low aflatoxin contamination:
  - Max contamination at 20 µg / kg would give an estimated 0.041 cancers / year / million people, i.e. reducing number by 59 cancers / year per 1000 million people*
  - Max contamination at 10 µg / kg would give an estimated 0.039 cancers / year / million people, i.e. reducing number by just 2 cancers / year per 1000 million people compared with 20 µg / kg
- In areas with high levels of hepatitis infection and high aflatoxin contamination
  - Max contamination at 20 µg / kg would give an estimated 1.7 cancers / year / million people, i.e. reducing number by 1300 cancers / year per 1000 million people*
  - Max contamination at 10 µg / kg would give an estimated 1.4 cancers / year / million people, i.e. reducing number by just 300 cancers / year per 1000 million people compared with 20 µg / kg

* Compared to mean level in abscence of a standard for maximum permissible contamination.

reduced. The risk manager will be looking for answers to a number of questions, such as:

- Does the risk assessment provide sufficient information to support a regulatory decision?
- What is the range of uncertainty in the data on exposure and health effects? Currently, methods of uncertainty analysis are being developed to better inform the risk manager.
- What were the key assumptions made during the risk assessment, and how would the outcome have differed if these assumptions had been different?
- What is the degree of confidence in the existence of the risk and the magnitude of the risk estimate?
- What is the range of biological variability with respect to susceptibility in the human population and different subpopulations, and the resistance or infectivity of the hazards?
- Are there population subgroups at increased risk of exposure and/or greater sensitivity to the effects of the exposure?

Overall, the confidence in the risk assessment will depend on the variability, uncertainty and assumptions identified in all the previous stages, and the weight of evidence and the type of data (human, animal, modelling or a description of the supply chain) on which it is based. Uncertainty is determined by the current state of knowledge and may be lessened by future advances in scientific understanding. Variability is a measure of inherent differences that cannot be reduced, but itself may be subject to uncertainty. If the degree of confidence in the risk assessment is low, the preferred risk management option may be to err on the side of caution. However, this is a risk management tool and is not based on scientific principles. Elimination of the hazard (zero tolerance) may be the preferred risk management option, but is not a realistic goal for many microbial hazards and chemical contaminants. It may be feasible to discontinue usage of some food additives. However, such decisions should also take into account whether there are suitable alternatives for the additive in question, or whether discontinuing its use could potentially lead to other adverse effects. For example, discontinuation of a preservative may increase the likelihood that microbial growth could result in harmful levels of microbial contamination.

## BOX 5C

### *Risk characterisation* – L. monocytogenes

**Individual cumulative risk of listeriosis:**

- Estimated from the probability of illness linked to consumption of a single serving, the number of servings per person per year, the proportion of Listeria strains that are virulent and the probability of illness at a given dose
- Annual cumulative risk ranged from 2 to 64 per billion for low risk populations and 1000 to 72,000 per billion for high risk populations

**Estimated incidence of listeriosis in a country of 50 million inhabitants:**

- Mean number of clinical cases of listeriosis estimated to be 57
- Mean number of deaths estimated to be 12

**Effect of eliminating milk from cows infected with *L. monocytogenes*:**

- Estimates reduced to 11 cases and 2 deaths per 50 million inhabitants

# RISK ASSESSMENT AS A SUPPORTIVE TOOL FOR THE RISK MANAGEMENT OF FOOD AND WATER

Risk assessment provides the basic information upon which risk management decisions are made, and the principles of risk assessment are generally defined by government departments. The risk assessment is usually conducted by expert committees, on behalf of the government departments, particularly with respect to defining acceptable levels of chemicals in food and drinking water. For microbiological agents, a risk assessment may be conducted by a government for policy-making purposes. It could also be conducted by the food industry during the different stages of food processing and production to minimise the potential for contamination and proliferation as part of the hazard analysis in the context of the HACCP system. The risk assessment aims to set out, in clear and transparent terms, the hazards and levels of risk that have been estimated to be associated with different exposure scenarios, and the uncertainties involved in those estimates. It may provide a basis for possible options for risk management and provide them with a scientific basis (as illustrated in Boxes 6A-C). Ideally, the outcome of the risk assessment would be pertinent to all geographical regions. This is generally not achievable because there are too many uncertainties, and it is therefore more normal to focus on a specific area or population.

The risk manager can then make policy decisions taking into account social demand and political and economic constraints on a regional basis. The impact of the resulting risk management strategy may then be monitored, both for the effectiveness of the control measures and to check that the controls have the health benefits indicated by the risk assessment, and on a time-scale pertinent to the disease in question. Thus, for control of microbiological agents, the benefits may be seen soon after control measures become effective, whereas for cancer-causing agents, it may take 10-30 years after the exposure has ceased for the associated cancer-rates to decrease.

In addition, advances in scientific understanding may lead to reductions in the uncertainties associated with the initial risk assessment. For agents of particular concern, it is therefore appropriate to consider periodic review, whether initiated by the scientific experts on the basis of new data, or by the risk manager on the basis of monitoring and surveillance data.

## BOX 6A

### Risk assessment as a supportive tool for risk management – the pesticide

**Other factors to take into account:**

- Availability of alternative pesticides for the specific application
- Economic aspects in different regions

**Risk management options might include:**

- Ban usage if alternatives are available
- Permit restricted usage to limit exposure

## BOX 6B

### *Risk assessment as a supportive tool for risk management – aflatoxins*

**Overall conclusions:**

- If only a small proportion of the food supply is contaminated, imposing maximum contamination levels would reduce intake in a small minority of people and therefore would not significantly affect overall cancer rates
- If a substantial proportion of the food supply is contaminated, reducing levels of contamination would reduce intake to large numbers of people and may result in detectable decreases in overall cancer rates

**Risk management options might include:**

- Vaccination against hepatitis B to reduce cancer potency of aflatoxin
- Improve farming and storage measures to reduce likelihood of fungal contamination
- Enforce standards for levels of aflatoxin in food

## BOX 6C

### *Risk assessment as a supportive tool for risk management –* L. monocytogenes

**Overall conclusions:**

- The risk assessment is based on a number of assumptions relating to milk production, the cheese-making process, consumption of raw milk cheese and the models used in mathematical analyses
- Results of the risk assessment model could be compared with epidemiological evidence in order to estimate the proportion of listeriosis that could be due to consumption of raw milk cheese in relation to other possible causes
- The model allowed for investigation of risk management options

**Risk management options might include:**

- Elimination of milk from cattle with *L. monocytogenes* mastitis
- Improved hygiene to reduce environmental contamination
- Advice to high risk populations

## SUMMARY

Risk assessment is a process that provides the scientific basis for decisions aimed at ensuring, maintaining and enhancing the safety of food and drinking water. The results of the risk assessment contribute to policy decisions on management and control of risk, and therefore the purpose of the risk assessment should be defined to answer the questions that will be ultimately posed by risk managers. Risk assessment consists of four defined stages: hazard identification, hazard characterisation, exposure assessment and risk characterisation.

For a new chemical under development, such as a new pesticide, hazard identification will commence with investigation of its biological properties under experimental conditions, according to internationally agreed procedures. Chemical substances that are naturally occurring, or have been in common usage for many decades, will usually not have been subjected to the same test strategies. However, where such a substance is the focus of human health concerns, there may be considerable data available in the scientific literature based on both experimental and epidemiological studies. Similarly, literature or public health data may support identification of pathogenic microbiological agents and definition of their hazardous properties.

Hazard characterisation 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 a toxic chemical) or estimation of the risk of disease associated with different doses (e.g. for microbiological agent and for cancer-causing chemicals). Exposure assessment aims to estimate possible intakes of the agent in different populations, and under possible different supply chain and consumer use scenarios. Risk characterisation then combines this information in order to estimate the possible risks, or establish if a particular use or product design can be considered acceptable, i.e. unlikely to cause harm. The risk characterisation aims to define the risks under the actual conditions of use and production examined by the exposure assessment, and estimate the potential health benefits of control measures, clearly stating confidence in the results and the sources of uncertainty. The risk manager can then consider this information in view of social and economic factors in order to make the most effective decisions on control of risks.

# GLOSSARY

**Acceptable Daily Intake (ADI):** Estimate of the amount of a substance in food or drinking water, expressed on a body mass basis (e.g. mg or µg/kg body weight), which can be ingested daily over a lifetime by humans without appreciable health risk.

**Acute toxicity:** Adverse effects occurring within a short time (usually up to 14 days) after administration of a single dose of test substance, or after multiple doses administered within 24 hours.

**Adverse effect:** Change in morphology, physiology, growth, development or lifespan of an organism which results in impairment of functional capacity or impairment of capacity to compensate for additional stress or increase in susceptibility to the harmful effects of other environmental influences.

**Carcinogen:** Agent (chemical, physical or biological) which is capable of increasing the incidence of malignant neoplasms (commonly referred to as cancer).

**Chronic toxicity:** Adverse effects following continued exposures over an extended period of time.

**Detoxication:** Process(es) of chemical modification which are usually catalysed by enzymes and which reduce or abolish the toxicity of a molecule.

**Effect:** A biological change in an organism, organ or tissue.

**Exposure:** Concentration or amount of a particular chemical agent, toxin or infectious microorganism that reaches the target population, organism, organ, tissues or cell, usually expressed in numerical terms of substance concentration, duration and frequency.

**Genotoxicity:** Ability to cause damage to genetic material. Such damage may be mutagenic and/or carcinogenic.

**HACCP:** Hazard Analysis Critical Control Point. A system that identifies, evaluates and controls hazards that are significant for food safety.

***in vitro*:** Literally "in glass" referring to a study in the laboratory usually involving isolated organ, tissue, cells or biochemical systems.

***in vivo*:** In the living body, referring to a study performed on a living organism.

**Infection:** colonisation by a microorganism.

**Infectious illness:** colonisation by a pathogenic microorganism leading to overt symptoms of disease.

**Infectious pathogen:** a microorganism that causes disease in humans by growth in the gut or in tissues.

**Long-term toxicity study:** A study in which animals are observed during the whole life span (or the major part of the life-span) and in which exposure to the test material takes place over the whole observation time or a substantial part thereof. The term chronic toxicity study is used sometimes as a synonym for long-term toxicity study.

**Maximum Residue Limit (MRL) for pesticide residues:** Maximum contents of a pesticide residue recommended by Codex to be legally permitted in or on food commodities and animal feeds. MRLs are based on data obtained following good agricultural practice.

**No-observed-adverse-effect-level (NOAEL):** The greatest concentration or amount of an agent, found by study or observation, that causes no detectable adverse alteration of morphology, functional capacity, growth, development or life-span of the target.

**No-observed-effect-level (NOEL):** The greatest concentration or amount of an agent, found by study or observation, that causes no detectable alteration of morphology, functional capacity, growth, development or life-span of the target.

**Pathogenic:** Capable of causing disease.

**Provisional Maximum Tolerable Daily Intake (PMTDI):** The end-point used for contaminants with no cumulative properties. Its value represents permissible human exposure as a result of the natural occurrence of the substance in food and in drinking water. For trace elements that are both essential nutrients and unavoidable constituents of food, a range is expressed, the lower value representing the level of essentiality and the upper value the PMTDI.

**Provisional Tolerable Weekly Intake (PTWI):** The end-point used for food contaminants such as heavy metals with cumulative properties. Its value represents permissible human weekly exposure to those contaminants unavoidably associated with the consumption of otherwise wholesome and nutritious food.

**Safety factor:** A factor applied to the no-observed-effect-level to derive an ADI. The value of the safety factor depends on the size and type of population to be protected and the quality of the toxicological information available.

**Short-term toxicity study:** An animal study (sometimes called a subacute or subchronic study) in which the effects produced by the test material, when administered in repeated doses (or continuously in food or drinking water) over a period of about 90 days, are studied.

**Toxin:** a harmful chemical produced by a micro-organism during the course of its growth.

**Uncertainty factor:** An alternative description of safety factor, which is being used increasingly because it indicates that the factor is to allow for uncertainties in the risk assessment process.

**Virulent:** Extremely infective and / or harmful.

# FURTHER READING

More details of the principles and methodology described in this monograph may be found in:

Buchanan, R.L., Smith, J.L. and Long, W. (2000). Microbial risk assessment: dose-response relations and risk characterization. *Int J Food Microbiol*. **58**(3): 159-72.

Codex Alimentarius Commission (1998). Draft principles and guidelines for the conduct of microbiological risk assessment. FAO/WHO document CX/FH 97/3, July 1998.

ICMSF (1998). Potential application of risk assessment techniques to microbiological issues related to international trade and food products.

ILSI Europe (1997). A Simple Guide to Understanding and Applying the Hazard Analysis Critical Control Point Concept. *ILSI Europe Concise Monograph Series*, 13 pp.

ILSI Europe (2000). The Acceptable Daily Intake – A Tool for Ensuring Food Safety. *ILSI Europe Concise Monograph Series*, 38 pp.

IPCS (1987). Environmental Health Criteria **70**. Principles for the Safety Assessment of Food Additives and Contaminants in Food. Geneva, World Health Organisation, International Programme on Chemical Safety, 174 pp. [NB. This volume is no longer in print, but the contents may be viewed on the IPCS web site at www.who.int/pcs/].

IPCS (1990). Environmental Health Criteria **104**. Principles for the Toxicological Assessment of Pesticide Residues in Food. Geneva, World Health Organisation, International Programme on Chemical Safety, 117 pp.

IPCS (1994). Environmental Health Criteria **170**. Assessing Human Health Risks to Chemicals. Geneva, World Health Organisation, International Programme on Chemical Safety, 73 pp.

IPCS (1999). Environmental Health Criteria **210**. Principles for the Assessment of Risks to Human Health from Exposure to Chemicals. Geneva, World Health Organisation, International Programme on Chemical Safety, 73 pp.

Kroes, R., Galli, C., Munro, I., Schilter, B., Tran, L.-A., Walker, R. and Würtzen, G. (2000). Threshold of Toxicological Concern for chemical substances present in the diet: a practical tool for assessing the need for toxicity testing. *Food and Chemical Toxicology*. **38**: 255-312.

Lammerding, A.M. and Fazil, A. (2000). Hazard identification and exposure assessment for microbial food safety risk assessment. *Int J Food Microbiol*. **58**(3):147-57.

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