

The evolution of microbiological risk assessment in food production¹

S. Notermans¹⁾, A.W. Barendsz and F. Rombouts
Foundation Food Micro & Innovation
Obrechtlaan 17, 3723 KA Bilthoven, The Netherlands
Email: foodmicro@planet.nl

Contents

List of abbreviations

General

- 1 Historical aspects of safe food production**
- 2 Evolution of food safety systems**
 - 2.1 The 'prohibition' principle
 - 2.2 The 'precautionary' principle
 - 2.3 Establishing process criteria
 - 2.4 Microbiological examination of food
 - 2.5 Introduction of Good Manufacturing Practices and Hazard Analysis Critical Control Points
 - 2.6 Predictive modelling
 - 2.7 Introduction of quantitative risk analysis
- 3 Microbiological risk assessment international food safety standards and future trends**
 - 3.1 Setting of current international standards
 - 3.2 International criteria: Future trends
- 4 Present and future uses of microbiological risk assessment in the food industry**
 - 4.1 Trends in food safety control
 - 4.2 Some examples
 - 4.2.1 Setting storage criteria for pasteurised milk
 - 4.2.2 From targets to HACCP criteria
- 5 Current issues in microbiological risk assessment**
- 6 References**

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List of abbreviations

ADI	Acceptable Daily Intake
ALARA	As Low as Reasonable Achievable
ALOP	Appropriate Level of Protection
CAC	Codex Alimentarius Commission
CCFAC	Codex Commission for Food Additives and Contaminants
CCFH	Codex Commission for Food Hygiene
CCP	Critical Control Point
CCPR	Codex Commission for Pesticide Residues
CCRVD	Codex Commission for Residues of Veterinary Drugs in Food
FAO	Food and Agriculture Organisation of the United Nations
FDA	Food and Drug Administration
FSO	Food Safety Objective
GAP	Good Agricultural Practice
GMP	Good Manufacturing Practice
HACCP	Hazard Analysis Critical Control Points
HTST	High-Temperature, Short-Time
IAMFES	International Association of Milk, Food and Environmental Sanitarians
IAFP	International Association of Food Protection
ICMSF	International Commission on Microbiological Specification for Foods
JECFA	Joint FAO/WHO Expert Committee on Food Additives
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
MRA	Microbiological Risk Assessment
MRL	Maximal Residue Level
NACMCF	US National Advisory Committee on Microbiological Criteria for Foods
PMTDI	Provisional Maximal Tolerable Daily Intake
PTWI	Provisional Tolerable Weekly Intake
QRA	Quantitative Risk Analysis
SPS Agreement	WTO Agreement on the Application of Sanitary and Phytosanitary Measures
WHO	World Health Organisation
WTO	World Trade Organisation

General

MRA is a relatively new tool in the quest for better means of ensuring the production of safe food. MRA comprises four successive key steps (i) hazard identification, (ii) hazard characterisation, (iii) exposure assessment and (iv) risk characterisation. The use of risk assessment ensures that control of food safety is based on a logical and scientific approach to the problems involved. In practice, elements of MRA have been utilised for many years, although, in earlier times, they were not formally recognised as such. Hazard identification, for example, began at the end of the nineteenth century when the work of van Ermengen served to clarify the etiology of botulism in man (Ermengem, 1896). Later milestones in this category included the recognition of *Clostridium perfringens* as a foodborne pathogen in 1943 (McClane, 1979) and *Bacillus cereus* in the 1950s (Granum, 1997). Human infections with *Listeria monocytogenes* were well known by the 1940s and foodborne transmission was suspected (Rocourt and Cossart, 1997), but it was not until the occurrence of an outbreak in Canada in 1981 that proper evidence was obtained. In this case, illness followed the consumption of contaminated coleslaw (Farber and Peterkin, 2000). Since then, numerous foodborne outbreaks have been reported in different countries, and prevention of listeriosis has become a major challenge for the Food Industry.

Regarding hazard characterisation, data have been obtained from the analysis of many incidents of foodborne disease. Although such information is not sufficient to establish dose-response relationships, some outbreaks have yielded useful data on attack rates and exposure levels for particular pathogens.

Even in the distant past, there was evidence of a rational approach to the control of food safety. Therefore, the evaluation of MRA in the present chapter begins with some historical aspects of safe food production, followed by discussion of food control systems that have been developed and applied in the past, with special reference to MRA principles. Section 3 deals with the establishment of international food safety standards based on the use of risk assessment. In Section 4, consideration is given to the ways in which MRA is becoming integrated in food industry practices and some examples of beneficial applications are included. Finally, current issues in MRA are discussed.

1 Historical aspects of safe food production

The need to produce safe food has a long history. Problems with foodborne diseases must have been a continuous preoccupation of early humans, once they began their hunting and food-gathering activities, and domestic production of food animals and crops. Although the exact timing is uncertain, organised food production probably started between 18,300 and 17,000 years ago, when barley production is said to have flourished in the Egyptian Nile Valley (Wendorf et al, 1979). During that time, there was a need to preserve the grain and keeping it in a dry condition was an obvious precaution. Attempts to preserve other foods were based mainly on experience gained in associating the spoilage of a food with the manner in which it had been prepared and stored. The same would be true for keeping food safe. Increasingly, it became clear that a safe condition could only be maintained if the product was kept dry and away from contact with air. Some foods were treated with honey and later with

olive oil (Toussaint – Samat, 1992). This led to the development of additional preservative measures, such as heating and salting. Once the preservative capability of salt was discovered, its value increased, since it was not available in sufficient quantity to meet the demand. According to Toussaint-Samat (1992), the large amount of salt in the Dead Sea was one of the reasons for the interest of the Romans in Palestine.

Over many millennia, mankind has learned how to select edible plant and animal species, and how to produce, harvest and prepare them for food purposes. This was mostly done on the basis of trial and error and from long experience. Many of the lessons learned, especially those relating to adverse effects on human health are reflected in various religious taboos, which include a ban on eating specific items, such as pork, in the Jewish and Muslim religions (Tannahill, 1973). Other taboos showed a more general appreciation of food hygiene. In India, for example, religious laws prohibited the consumption of certain ‘unclean’ foods, such as meat cut with a sword, or sniffed by a dog or cat, and meat obtained from carnivorous animals (Tannahill, 1973). Most of these food safety requirements were established thousands of years ago when religious laws were likely to have been the only ones in existence. The introduction of control measures in civil law was of a much later date.

Because the underlying causes of foodborne illness were unknown, microbial food poisoning was recurrent. However, the situation changed after 1795, when the French government, driven by war, offered a substantial reward for anyone developing a new method of preserving food. It was Nicholas Appert, a Parisian confectioner, who accepted the challenge and developed a wide-mouth glass bottle that was filled with food, corked and heated in boiling water for about six hours. In 1810, Durand in England patented the use of tin cans for thermal processing of foods, but neither Appert nor Durand understood why thermally processed foods did not spoil (Hartman, 1997), despite the fact that in 1677 van Leeuwenhoek had discovered ‘his little heat-sensitive animalcules’ (Dobell, 1960).

It was Louis Pasteur who provided the scientific basis for heat preservation in the period 1854-1864. During this time, he showed that certain bacteria were either associated with food spoilage or caused specific diseases. Based on Pasteur’s findings, commercial heat treatment of wine was first introduced in 1867 to destroy any undesirable microorganisms, and the process was described as ‘pasteurisation’. Another important development occurred in Germany, when Robert Koch introduced a method of growing microorganisms in pure culture and, with colleagues, first isolated the cholera vibrio in 1884, during a worldwide pandemic (Chung et al., 1995). Over the next 100 years or more, laboratory isolation and study of pure cultures of microbes remained among the predominant activities of food microbiologists (Hartman, 1997).

2. Evolution of food safety systems

When it was accepted that people can contract disease from contaminated food, hygiene control laws were introduced and examples can be seen in old legal records. Table 1 gives an overview of the more important milestones in developing food safety systems. In the absence of knowledge about the causes of serious foodborne diseases and their etiology, use was made of the ‘prohibition’ principle. This means that it was prohibited to produce and/or to consume certain types of food after it was realised that the foods could be a cause of high mortality. The principle was used particularly to protect special groups of individuals within society,

such as soldiers. After the recognition at the end of the nineteenth century that microbial agents were often responsible for foodborne illness, systems for controlling the safety of the food supply began to be introduced.

Table 1. Important milestones in the development of food safety systems

Time	Activity
Distant past	Use of 'prohibition' principle to protect special groups within society against foodborne illnesses
1900 – until now	Microbiological examination of food
1922	Introduction of process performance criteria by Esty & Meyer for canned, low-acid food products
1930-1960	Use of risk assessment (for different pathogenic organisms) in setting process performance criteria for heat pasteurisation of milk
1960	Introduction of good manufacturing practices
1971	Introduction of formal hazard analysis critical control point system
ca 1978	Start with predictive modelling of bacterial growth in food
1995	Formal introduction of formal quantitative risk analysis

First, use was made of microbiological testing of foods and this became widely accepted as a means of assessing food safety during the early part of the twentieth century. Eventually, statutory microbiological requirements relating to food safety were established in many parts of the world. Further progress occurred when Esty and Meyer (1922) developed the concept of setting process performance criteria for heat treatment of low-acid canned food products to reduce the risk of botulism. Later, many other foods processed in this way were controlled in the same manner. An outstanding example is the work of Enright et al. (1956, 1957) who established performance criteria for the pasteurisation of milk that provided an appropriate level of protection against *Coxiella burnetii*, the causative agent of Q Fever. Studies for tuberculosis have been carried out earlier. The work is an early example of the use of risk assessment principles in deriving process criteria.

With greater knowledge of the more important foodborne diseases and establishment of risk factors from analyses of outbreaks, came the development of more comprehensive means of controlling food safety in production. These included the elaboration of GMP, which help to minimise microbial contamination of food from personnel and the production environment, and, ultimately, the HACCP system (Department of Health, Education and Welfare, 1972), in which GMP play an important part.

The ability of different bacteria to multiply in foods is influenced by several key factors, including pH, water activity and storage temperature. The effects of these factors, both singly and in combination, have been studied extensively in laboratory media and model food systems, and this has led to the development of mathematical models for predicting bacterial growth in commercial food products. Although not a food safety system on its own, predictive modelling is a valuable tool, which has helped to make possible the introduction of QRA. The latter has been used for many years in other disciplines and its use in food microbiology has been stimulated by the decision of the World Trade Organisation (WTO) to

promote free trade in safe food (Anon, 1995). It has been emphasised, however, that control of food safety in this context must be based on the application of sound scientific principles, and risk analysis is seen as the basis for ensuring that the requirement is met.

The next sub-section gives more detailed information on the above-mentioned food safety initiatives, with special reference to risk assessment procedures.

2.1 The 'prohibition' principle

As their trade in food increased, the Romans paid greater attention to the question of preventing spoilage, and a new rule emerged: it was prohibited to sell spoiled food of any kind. The aedilis (churchwarden) inspected and controlled food markets, and was charged with confiscating any food that had become spoiled. Over the last 2000 years, the 'prohibition' principle has continued to be applied in many societies to protect consumers from both spoiled food and that likely to contain deadly disease agents. Some examples are given below.

Consumption of blood products. In an historical account of food safety measures, Baird-Parker (2000) describes the action taken by Emperor Leo VI of Byzantium (886 - 911 AD). The Emperor introduced an outright ban on the consumption of blood products as a means of reducing the high incidence of poisoning associated with sausages among his people. The law applied particularly to blood sausages and carried a high penalty if it was disregarded, which indicates the seriousness of the problem. It was stated: 'A person found to have blood prepared as food, whether he buys or sells it, shall have all his property confiscated and, after having been severely scourged and disgracefully shaved, shall be exiled for life.' From the data available and current expert knowledge, it is clear that the 'blood disease' was actually botulism.

Selling of contagious flesh. In a document entitled 'A history of government regulation of adulteration and misbranding of food,' Hutt and Hutt (1984) refer to the English Statute of Pillory and Thumbrell (1266/67). This required the following: 'If any butcher do sell contagious flesh or which has died from the murrain (rinderpest), he must be punished.' The customary punishment was to be placed in the stocks with the offending meat buried underneath.

Unsold fish. In 1319, the municipal authorities in Zurich, Switzerland issued an ordinance prohibiting the sale of any fish that had been left over from the day before. A similar rule also operated in the city of Basel. However, such fish, could still be sold to strangers (Kampelmacher, 1971).

Eating of pufferfish. In Japan, a dish known as 'fugu' is, historically, one of the most favoured and heraldous forms of fish eating. Nevertheless, consumption of this food has resulted in many deaths, and the problem continues to this day. Consumption of the delicacy was banned in 1550 by the Emperor, after a group of soldiers had died, but the ban was abolished in 1888 when the Japanese Prime Minister tasted a small sample of fugu and survived. This disease is known as blowfish or pufferfish poisoning and is due to the neurotoxic effects of the tetrodotoxin, which occurs in various species of pufferfish. The dish is now prepared only by chefs that have been specially trained and certified by the Japanese

government and can be relied upon to free the flesh of the toxic liver, gonads and skin. Despite these precautions, many cases of tetrodotoxin poisoning are reported each year in people consuming fugu (Source: Medical Journal, 12 June, 2001, vol.2, no.6).

Sale of bongkrek. In the Regency of Banyumas and surrounding areas of Central Java, Indonesia, tempe bongkrek and other coconut-based products are prepared from partly defatted coconut. The raw material for tempe bongkrek is sometimes mixed with the residue obtained from the manufacture of tofu (soybean curd) and allowed to ferment with the mould *Rhizopus oligosporus*. Under certain conditions, a contaminating bacterium, *Burkholderia cocovenans*, is able to grow and produce two distinct toxins: colourless bongkrek acid and yellow-coloured toxoflavin. Bongkrek food poisoning usually has a latency period of 4-6 hours. Typical symptoms include malaise, abdominal pain, dizziness and extensive sweating. The victim becomes fatigued and drowsy and eventually passes into a coma. Death occurs 1-20 hours after the onset of the initial symptoms (Steinkraus, 1996). Because many Banyumas people have died as a result of eating tempe bongkrek, sale of the product is now prohibited.

2.2 The ‘precautionary’ principle

Once proper scientific data became available, the principle of prohibition began to be largely replaced by food safety regulations, which included process performance criteria, product specifications and specified storage conditions. The risk of botulism from blood sausages was minimised by introducing both product specifications and requirements for storage. As mentioned previously, the safety of fugu was improved by giving more attention to the training of chefs and ensuring that toxic organs were properly removed from the fish.

Despite these advances, another principle, the ‘precautionary’ principle, is still relevant in some situations, although its application is mainly restricted to certain vulnerable groups of the population, where absolute safety cannot be guaranteed with respect to some foods. For example, senior citizens in the USA are advised not to eat the following types of food (see www.foodsafety.gov/~fsg/sr2.html):

- Raw fin fish and shellfish, including oysters, clams, mussels and scallops.
- Raw or unpasteurised milk or cheese.
- Soft cheese, such as feta, brie, camembert, blue-veined and Mexican-style cheese.
- Raw or lightly cooked eggs or egg products, including salad dressings, cookie or cake batter, sauces and beverages, such as egg nog. (Foods made from commercially pasteurised egg are safe).
- Raw meat or poultry.
- Raw sprouts (alfalfa, clover and radish).
- Non-pasteurised or untreated fruit or vegetable juice. (These juices will carry a warning label).

The reason for giving such advice to the elderly is that they are more likely to be affected by any harmful bacteria that are present in the above foods. Once illness occurs, older people face the risk of more serious health problems, even death. With increasing age, natural defences, such as the immune system and production of stomach acid, become weaker. Also, underlying conditions, including diabetes and kidney disease, as well as some cancer treatments, may increase the risk of an individual succumbing to foodborne illness and

suffering serious consequences. Other groups within the population may also show greater susceptibility to foodborne illness. These include pregnant women, neonates and patients given immunosuppressive drugs for treatment of diseases such as cancer and rheumatoid arthritis. Here, examples of appropriate precautionary advice include a recommendation to avoid feeding honey to infants below one year of age, because of the risk of botulism (described in detail by Lund and Peck, 2000). Also advice is given in several countries to pregnant women to stop eating certain pates and soft cheeses due to the risk of contracting listeriosis.

Unfortunately, the warning of vulnerable groups against these particular hazards varies considerably between countries and in some cases is non-existent. At the other end of the 'precautionary' scale, is the use of it in risk management when there is a lack of proper scientific evidence or possible legal difficulties. A recent example from the UK was the ban on butchers selling beef-on-the-bone, due to the perceived risk of transmitting to humans the agent of bovine spongiform encephalopathy from bone marrow. Although the risk was considered extremely small, sale of the product was nevertheless prohibited by law.

2.3 Establishing process criteria

At the start of the twentieth century, it was already recognised that protection of the public against foodborne hazards required proper control of heat treatments used commercially in food production. Two examples are presented here: (i) the performance criteria for destroying spores of *Clostridium botulinum* in low-acid, canned foods (Esty and Meyer, 1922) and (ii) the process criteria for *Coxsackie burnetii* in milk pasteurisation, as determined by Enright et al. (1957).

Setting of process performance criteria for heat treatment of low-acid canned foods.

The first mathematical evaluation of the heat sterilisation process for canned foods was made by Bigelow et al. (1920) and later developed by Ball (1923) to derive methods for calculating the times necessary to process canned foods at appropriate temperatures. For commercial sterilisation, the goal of thermal processing was to reduce the probability of survival and growth of microorganisms in a particular canned food to an acceptably low level. The starting point for the rationale of what is now termed 'an appropriate level of protection' (ALOP) was the work of Esty and Meyer (1922). They derived process performance criteria for the destruction of spores of proteolytic strains of *Clostridium botulinum* in low-acid canned foods. It was proposed that requirements for sterilisation should be based on (i) the response to heating of the most heat-resistant spores found among strains of *Clostridium botulinum* and (ii) a reduction in the spore population by a factor of 10^{11} - 10^{12} to ensure the desired level of product safety. For this purpose, heat inactivation trials were carried out on 109 different strains of the test species. The resultant performance criteria, based on the approach outlined above, have been applied over many years and have proved to be sound, with an adequate margin of safety (Pflug and Gould, 2000).

Process performance criteria for heat pasteurisation of milk. The work of Enright et al. (1957) led to the development of process standards for controlling *Coxsackie burnetii* in milk. The heat treatments used initially for milk were designed to inactivate any tubercle bacilli present and these were considered to be the most heat-resistant of the nonsporulating pathogenic bacteria likely to occur in the product. The treatments were based on information from many studies

on the heat-resistance of both human and bovine strains (*Mycobacterium tuberculosis* and *Myc. bovis* respectively). In the USA, the heating regime adopted in 1924 for the conventional process was 142°F (61.1°C) - 145°F (62.8°C) for 30 min. In 1933 a heating regime was introduced for the High-Temperature, Short-Time (HTST) process: 161°F (71.7°C) for 15 s.

In practice, *Cox. burnetii* appears to be slightly more heat-resistant than the tubercle bacilli and, following recognition that the organism, which causes Q fever in man, could be transmitted by raw milk, it was necessary to check on the adequacy of existing pasteurisation processes for inactivating the organism. The work undertaken by Enright and colleagues (1956, 1957) fulfilled this requirement and, although no formal MRA was employed, elements of the MRA approach were implicit in their studies. These aspects are discussed below.

The organism and the disease it causes: *Cox. burnetii* is a small, Gram-negative bacterium, originally classified as a rickettsia, that cannot be grown in axenic culture but can now be cultivated *in vitro* in various cell lines (Maurin and Raoult, 1999). Q fever is characterised by fever, chills and muscle pain, with occasional long-term complications. It was first described by Derrick (1937) and is known to occur worldwide. The organism infects many wild and domestic animals, which often remain asymptomatic. Domestic animals, such as cattle, sheep and goats, are considered the main sources of infection for humans (Maurin and Raoult, 1999) and, when shed in milk, *Cox. burnetii* is often present in relatively high numbers.

Hazard identification: contact with infected animals was known to result in transmission of *Cox. burnetii* to man, with subsequent development of illness, and the likelihood of the organism contaminating raw milk was recognised. Early on, there was a lack of epidemiological evidence for transmission via milk, but this was suspected in several outbreaks and there was strong supporting evidence from a UK outbreak in 1967 (Brown et al. 1968). Thus, the hazard was the presence of *Cox. burnetii* in milk intended for human consumption.

Dose response: there was no information on the dose response in humans, since challenge trials had not been carried out and epidemiological data were lacking in this respect.

Exposure assessment: information relevant to this step in MRA was obtained by injecting guinea pigs to determine the presence and titre of *Cox. burnetii* in milk. The organism was found in 33% of 376 samples of raw milk from California, USA. “The maximum number of *Cox. burnetii* demonstrated in the milk of an infected dairy cow was the number of organisms contained in 10,000 infective guinea pig doses of *Cox. burnetii* per millilitre” (Enright et al., 1957). Similar titres were found in milk that had been frozen and thawed. However, the study did not involve testing of all breeds of dairy cattle, and it is possible that even higher levels of shedding may have occurred in some breeds that were not examined. Nevertheless, it was concluded that the maximum level of consumer exposure would be represented by the highest infective dose demonstrated in this study and that the pasteurisation process should bring about thermal inactivation of such a number (Enright et al., 1957).

Risk characterisation: the risk involved in consuming raw milk could not be estimated because of the absence of dose response data. The data for the prevalence of contaminated milk, the maximum level of contamination and the fact that milk would have been consumed

regularly by the majority of the population were probably implicit factors in an assumption that the risks associated with inadequate heat treatment were high.

The studies of Enright et al. (1956, 1957) led to the conclusion that heating at “143°F for 30 min was wholly inadequate to eliminate viable *Cox. burnetii* from whole, raw milk, while heating at 145°F ensures elimination of these organisms with a high level of confidence” (Enright et al., 1957). This led to the adoption of the higher temperature for vat pasteurisation in the USA. The work on the HTST process indicated that the recommended standard of 161°F for 15 s was sufficient for total elimination.

2.4 Microbiological examination of food

Microbiological testing, as a means of assessing whether a food product is hazardous due to the presence of pathogens, is of relatively recent origin. It became the vogue only after Robert Koch developed a method for growing microorganisms in pure culture and foodborne organisms capable of causing spoilage or disease were recognised and could be enumerated (Hartman, 1997). Over the last 80 years or so, many different methods have been devised for detecting pathogenic organisms and/or their toxins. Even from the beginning of that period, statutory microbiological requirements relating to food safety were established in many countries and were based on the testing of prepared foods for the organisms or toxins of concern.

A disadvantage was that routine examination of foods for a multiplicity of pathogens and toxins was impractical in most laboratories and an alternative approach was needed. This led to widespread use of microbial groups or species that were more readily detectable in foods and considered to be indicative of conditions in which the food had been exposed to contamination with pathogens, or been under-processed. Enumeration of the organisms was even used as a measure of the possible growth of pathogens in a food, should these have been present. The bacteria in question were termed ‘indicator organisms’ and they have value for indirect assessment of both microbiological safety and quality of foods. The use of indicator organisms flourished, especially in the period 1960-1980. During that time, numerous procedures for enumerating bacterial indicators were described (e.g. American Public Health Association, 1966; United States Food and Drug Administration, 1972). Clearly, the main objective of their use was to reveal conditions of food handling that implied a potential hazard. Furthermore, some indicators were proposed as a possible index rather than a mere indication of faecal contamination in food (Mossel, 1982).

Setting criteria. The traditional approach to controlling food safety has been based on education and training of personnel, inspection of production facilities and operations, and microbiological testing of the finished product. Testing of the product is usually an integral part of the overall control programme, and the perceived risk of foodborne illness from the presence of a particular pathogen is reflected in the limit values that are set for the organism in a specific type of food. Where possible, these criteria are based on epidemiological data and are a reflection of the minimum dose expected to cause illness. Table 2 gives some values that are essentially derived from analyses of outbreaks of foodborne disease. The data show a clear parallel between limit values and the minimum dose associated with human disease. In general, infective organisms like *Salmonella* should be absent from food because very low numbers are known to be capable of causing illness (D’Aoust, 1989). On the other

hand, toxigenic bacteria, such as *Staphylococcus aureus*, may be acceptable at levels that are well below those causing food to become hazardous. With foodborne intoxications caused by *Staph. aureus*, the numbers present in the food usually exceed 10^7 cfu per g (Bergdoll, 1989).

Table 2. Correlation between minimum dose considered to cause disease and criteria set for end-products

Pathogenic organism	Minimum dose considered to cause disease ¹⁾	Probability of infection from exposure to 1 organism ²⁾	General end-product criteria used ³⁾
Infectious organism			
<i>Shigella</i>	1	1.0×10^{-3}	Absence/ 25 gram
<i>Salmonella</i>	1	2.3×10^{-3}	Absence/ 25 gram
<i>Campylobacter</i>	1-10	7.0×10^{-3}	Absence/ 25 gram
<i>Listeria monocytogenes</i>	$> 10^3$		< 100/ gram
<i>Vibrio parahaemolyticus</i>	$> 10^4$		< 10^3 / gram
Toxico-infectious organisms			
<i>Clostridium perfringens</i>	$> 10^6$		< $10^5 - 10^6$ / gram
<i>Bacillus cereus</i>	$> 10^6$		< $10^5 - 10^6$ / gram
Organisms causing intoxication			
<i>Staphylococcus aureus</i>	$> 10^6$		< $10^5 - 10^6$ / gram

1) Based on analysis of foodborne disease outbreaks (presented in Doyle, 1989)

2) Rose and Gerba (1991)

3) *Criteria for pathogenic organisms are not yet well established and they may differ from country to country. The validity of the criteria starts mostly after production and ends at the time of consumption.*

Shortcomings of microbiological testing. Leaving aside questions regarding the accuracy and reproducibility of the methods used, it is clear that microbiological testing of food is of limited value without a sound sampling plan. To overcome the problem, a book on food sampling was produced by the International Commission on Microbiological Specifications for Foods (ICMSF, 1974). The book gives details of statistically-based sampling plans for the microbiological examination of different types of food.

Although the book gives an excellent account of the various sampling plans, it also reveals the limitation of testing for pathogenic organisms that may be infrequent, low in number and unevenly distributed throughout the test batch, especially when complete absence is the only acceptable result. Thus, testing to ensure that the target pathogen is absent from the batch requires uneconomically large numbers of samples, with no guarantee that absence of the organism can be established.

2.5 Introduction of Good Manufacturing Practices (GMP) and Hazard Analysis Critical Control Points (HACCP)

GMP

One of the first quality assurance systems developed by the food industry was that involving the application of GMP, as a supplement to end-product testing. GMP have been used for many years to ensure the microbiological safety and quality of food, and they provide a framework for hygienic food production. The establishment of GMP is the outcome of long practical experience and they include attention to environmental conditions in the food plant, e.g. requirements for plant layout, hygienic design of equipment and control of operational procedures. The GMP concept is largely subjective and qualitative in its benefits. It has no direct relationship with the safety status of the product. For these reasons, the concept has been extended by introducing the HACCP system, which seeks, among other things, to avoid reliance on microbiological testing of the end-product as a means of controlling food safety. Such testing may fail to distinguish between safe and unsafe batches of food and is both time-consuming and relatively costly.

HACCP

The HACCP concept is a systematic approach to the identification, assessment and control of hazards in a particular food operation. It aims to identify problems before they occur and establish measures for their control at stages in production that are critical to ensuring the safety of food. Control is proactive, since remedial action is taken in advance of problems occurring.

In a review of the historical background, Barendsz (1995) and Untermann et al. (1996) described the development of the HACCP approach, which began in the 1960s. The concept arose from a collaboration between the Pillsbury Company, the US Army Natick Research and Development Laboratories and the US National Aeronautics and Space Administration. The original purpose was to establish a system of safe food production for use in human space travel. At that time, the limitations of end-product testing were already appreciated and therefore more attention was given to controlling the processes involved in food production and handling. When first introduced at a congress on food protection (Department of Health, Education and Welfare, 1972), the concept involved three principles: *(i)* hazard identification and characterisation; *(ii)* identification of critical control points (CCPs) and *(iii)* monitoring of the CCPs.

Many large food companies started to apply HACCP principles on a voluntary basis, and in 1985 the US National Academy of Science recommended that the system should be used. Further support came from the ICMSF (1988), which extended the concept to six principles. They added specification of criteria, corrective actions and verification (see table 3). In 1989, the US National Advisory Committee on Microbiological Criteria for Foods added in a further principle: the establishment of documentation concerning all procedures and records appropriate to the principles and their application. Use of the HACCP system was given an international dimension by the Codex Alimentarius Commission (CAC) which published details of the principles involved in 1991 and their practical application (CAC, Committee on Food Hygiene (1991). In 1997, the CAC laid down the 'final' set of principles and clarified the precise meaning of the different terms (CAC, Committee on Food Hygiene, 1997):

- General principles of food hygiene (Alinorm 97/13, Appendix II)
- HACCP system and guidelines for its application (Alinorm 97/13A, Appendix II)

- Principles for the establishment and application of microbiological criteria for foods (Alinorm 97/13A, Appendix III)

The full HACCP system, as described in Alinorm 97/13, is shown in Table 3. The document also gives guidelines for practical application of the HACCP system. By 1973, the FDA had made the use of HACCP principles mandatory for the production of low-acid canned foods (FDA, 1973) and, in 1993, the system became a legal requirement for all food products in the European Union (Directive 93/43).

Table 3. The seven principles of the HACCP system, (CAC, Committee on Food Hygiene, 1997)

Principle	Activity
1 Conduct a hazard analysis	List all potential hazards associated with each step, conduct a hazard analysis, and consider any measures to control identified hazards
2 Determine the Critical Control Points (CCPs)	Determine Critical Control Points (CCPs)
3 Establish critical limit(s)	Establish critical limits for each CCP
4 Establish a system to monitor control of the CCP	Establish a system of monitoring for each CCP
5 Establish corrective actions	Establish the corrective action to be taken when monitoring indicates that a particular CCP is not under control
6 Establish verification procedures	Establish procedures for verification to confirm that the HACCP system is working effectively
7 Establish documentation and record keeping	Establish documentation concerning all procedures and records appropriate to these principles and their application

Despite widespread usage, the present HACCP concept still has some weak points. One of them is the definition of a hazard. This is not defined as “an agent with the potential to cause an adverse health effect”, as in risk assessment, but as “an unacceptable contamination, growth and/or survival by microorganisms of concern” (ICMSF, 1988), which is more restrictive and does not cover all possible hazards. Another weakness arises from the definition of a CCP. It is stated that a CCP is a location, practice etc where hazards can be minimised (ICMSF, 1988; International Association of Milk, Food and Environmental Sanitarians (IAMFES), 1991) or reduced to an acceptable level (Bryan, 1992; Alinorm 97/13). In both cases, these are qualitative objectives and may lead to differing interpretations. It was Notermans et al. (1995) who first made a plea to use the principles of quantitative risk assessment for setting critical limits at the CCPs (process performance, product and storage criteria). It was their opinion that only when the critical limits are defined in quantitative terms the level of control at the CCPs can be expressed realistically. At the

International Association of Food Protection (IAFP) meeting in 2001, Buchanan (2001) also favoured the use of these principles and suggested that food safety objectives should encompass end-product criteria, which are related to the criteria used in processing.

2.6 Predictive modelling

Modelling in food microbiology began about 1920, when methods were developed for calculating thermal death times. These models revolutionised the canning industry (Pflug and Gould, 2000). Later, Monod (1949, 1950) developed a model that described the continuous, steady-state culture of microorganisms and became the basis for continuous fermentation processes. In principle, the model was analogous to that used for chemical processes. The recent resurgence of predictive modelling in relation to microbial growth in food originated in the 1960s and has been reviewed by Ross and McMeekin (1994). In contrast to the situation studied by Monod, the identities and concentrations of nutrients involved are unknown and the organisms of interest are initially present in low numbers, with growth conditions often being less than optimal. For these reasons, initial attempts at mathematical modelling in food microbiology have been more empirical than was the case for fermentation processes, focussing on batch rather than continuous-culture kinetics. As shown by Whiting and Buchanan (1997), growth data are fitted to equations using interactive least-square computer algorithms. Assumptions about randomness, normal distribution and stochastic specifications are the same as they would be for any statistical application of regression (Ratkowsky, 1993). All models are actually simplifications that represent the complex biochemical processes controlling microbial growth and are limited to the most important input parameters, such as temperature, time, water activity and pH. One of the reasons for simplifying the approach is that knowledge of the complex biochemical processes involved is far from complete. The big advantage is that the current models are easy to handle; however, the outcome should always be used with caution and verification may be necessary in some cases.

Primarily, the development of predictive modelling was driven by the proliferation of refrigerated and limited-shelf-life food products. It was recognised that (i) even so-called 'rapid' microbiological methods were too slow to show, within an acceptable period of time, whether microbes in the product grew or died (Spencer and Bains, 1964); (ii) testing of factors in a food product that affect microbial growth and toxin production, whether singly or in combination, is laborious and time-consuming and (iii) work had been done in Canada to draw together the results of numerous growth experiments on *Clostridium botulinum* (Hauschild, 1982). The mathematical and statistical tools already existed prior to the expansion in modelling activity and the process was favoured by the introduction of powerful personal computers and the availability of user-friendly software.

In the review of Ross and McMeekin (1994), the main reasons for developing predictive models were summarised as follows:

1. To permit predictions of product shelf-life and safety, and the consequences of changes in product formulation or composition; to facilitate a rational design for new processes etc; to meet or to obtain an insight into requirements for product safety or shelf-life.
2. To allow objective evaluations to be made of processing operations and, from this, an empowering of the HACCP approach.

3. To provide an objective evaluation of the consequences of any lapses in process control and subsequent storage of the end-product.

Now that MRA has become established in food microbiology, it is clear that the use of predictive models is essential in risk assessment. This is especially true for exposure assessment. In many foods, particularly those that are fresh and have a short shelf-life, rapid changes in microbial populations can occur and the models are needed to determine, for example, the necessary storage conditions. The models can also provide information about risk factors in handling the product, which have a considerable influence on human exposure to particular pathogens. They may also help to clarify the effects of different control options. Thus, the modelling approach facilitates control of the most important factors that affect food safety. Without the use of predictive models, a quantitative MRA for assessing food safety would be virtually impossible.

2.7 Introduction of QRA

Systematic risk analysis approaches have been used by the Food and Agriculture Organisation of the United Nations (FAO) and the World Health Organisation (WHO) since 1955, when the evaluation of food additives at the international level was initiated as a result of a joint FAO/WHO conference on food additives. The conference recommended to the Directors-General of FAO and WHO that one or more expert committees should be convened to address the technical and administrative aspects of chemical additives and their safety in food. This recommendation provided the basis for setting up the Joint FAO/WHO Expert Committee on Food Additives (JECFA). The JECFA started its meetings in 1956, initially to evaluate the safety of food additives.

Risk assessment has also evolved over the last decade within the CAC. The Commission, which was established in the 1962 under the parentage of FAO and WHO, is an intergovernmental organisation with the responsibility for developing international standards, guidelines or other recommendations for food in order to protect the health of consumers and facilitate international trade. In the course of time, the CAC has enlarged its activities and, in addition to risk evaluation for food additives, chemical contaminants, pesticide residues, and veterinary drug residues in foods, the issue of biological hazards in foods is now also being addressed. However, no clear MRA activities were undertaken prior to 1995.

The development of MRA was strongly stimulated when in 1995, at the GATT Uruguay Round, the WTO was established and a free trade in safe food was agreed. In the WTO Agreement on the Application of Sanitary and Phytosanitary Measures, the so-called SPS Agreement (Anon, 1995), requires that countries signatory to the agreement base their laws concerned with protecting human, animal and plant health on a risk analytical basis. Thus, the SPS Agreement requires food safety legislation to be scientifically based and the process of risk assessment to be applied, for example, when introducing microbiological criteria for controlling imported foods. In the pursuance of harmonisation and to avoid the need for all countries and all food producers to carry out a risk assessment on each of their products, the WTO SPS Agreement has chosen the scientifically-based international standards, guidelines and recommendations of three organisations, one of which is the CAC, as the preferred measures for adoption by WTO members. In addition, the SPS Agreement states that countries should take into account the risk assessment technique developed by the relevant

international organisations, when undertaking a risk assessment. As a result of this, FAO and WHO began to organise expert consultations dealing with food safety risk assessment, with the purpose of providing member countries with principles and guidelines for undertaking such an assessment. An overview of the documents produced is given briefly in Table 4.

Table 4. FAO/WHO documents dealing with food-related risk analysis

Year	Risk Analysis documents	References
1995	Application of risk analysis to food standards issues	FAO/WHO, 1995
1997	Risk management and food safety	FAO/WHO, 1997
1998	The application of risk communication to food standards and safety matters	FAO/WHO, 1998
1999	Risk assessment of microbiological hazards in foods	FAO/WHO, 2000a
2000	The interaction between assessors and managers of microbiological hazards in food	FAO/WHO, 2000b

The first expert consultation was devoted to the application of risk analysis to food safety standards issues. The consultation was convened at the request of the Forty-first Session of the CAC Executive Committee, with the aim of promoting consistency in the use of risk analysis for food safety purposes. The main objective was to provide FAO, WHO and CAC, as well as member countries, with advice on practical approaches for the application of risk analysis to food standards issues. At that meeting, food safety risk analysis terms were defined. A model for risk assessment was also agreed upon. This comprises the four components: (i) hazard identification, (ii) hazard characterisation, (iii) exposure assessment, and (iv) risk characterisation.

At that consultation, the estimation of risk from biological agents was considered in detail, since it was the general view of the experts that such risks are in many ways a much larger and more immediate problem to human health than risks associated with chemical contaminants in food.

At an expert consultation in 1997, a risk management framework was set up and general principles of food safety risk management were elaborated. In addition, key risk management terms were defined. The main elements of risk management were identified as (i) risk evaluation, (ii) assessment of risk management options (iii) implementation of management decisions and (iv) monitoring and review. As far as the general principles are concerned, it was stated that risk management decisions should be transparent, primarily aimed at the protection of human health and should ensure that the scientific integrity of the risk assessment process is maintained.

As a logical continuation, a third expert consultation dealt with the application of risk communication. The main issues addressed at this meeting were the principles of risk communication and barriers to, and strategies for, making the process effective. It is generally accepted that risk communication is essential throughout the risk analysis process. For successful risk communication, it is important that (i) all interested parties are involved, (ii) use is made of individuals trained in risk communication, (iii) it is ensured that risk

communication is received and understood, and (iv) transparency is fostered during the whole process. The nature of the risk and the benefits and uncertainty in risk assessment and assessment of risk management options are regarded as the main elements for effective risk communication. The main barriers in risk communication include differences in perception and receptivity, lack of understanding of the scientific process, and media and social characteristics.

The fourth expert consultation, convened in 1999, was directed specifically at risk assessment of microbiological hazards in foods. The main outcome of this consultation was an outline strategy and mechanism for addressing MRA at the international level. The expert consultation made recommendations regarding the activities required to support MRA and how to improve the necessary capabilities. In addition, it recommended that outcomes of risk assessments should be immediately integrated into HACCP plans, that additional expert meetings should be held and that collaborative studies should be conducted between developing and developed countries.

In 2000, a second specific expert consultation was organised, dealing with the interaction between assessors and managers of microbiological hazards in foods. At this consultation, the linkage between risk assessment and risk management was discussed in more detail, with the aim of providing guidance on how both processes could be improved. Issues addressed ranged from the identification of a food safety problem and the establishment of risk profiles to assessment of the effectiveness of management decisions. The former is of interest in relation to collecting as much information as possible for both risk assessment purposes and effective risk management.

Current microbiological risk assessment activities. In 1999, following the request of the CAC and in order to address the needs of their member countries, FAO and WHO initiated a series of joint expert consultations to assess risks associated with specific microbiological contaminants in foods. This followed the adoption by the CAC of the Principles and Guidelines for the Conduct of Microbiological Risk Assessment (MRA), elaborated by the Codex Commission for Food Hygiene (CCFH), (CAC/GL 30, 1999).

The aims of these joint expert consultations were to provide a transparent review of scientific data on the state of the art of MRA, and to develop the means of achieving sound quantitative risk assessments for specific pathogen-commodity associations. The work included an evaluation of existing risk assessments, a review of the available data and risk assessment methodologies, highlighting their strengths and weaknesses and how they might be applied, provision of examples and identification of information needs/gaps. A further aim of these consultations was the development of guidelines relating to the different steps in risk assessment, such as hazard characterisation and exposure assessment. The purpose of such guidelines would be to help the risk assessor, the risk manager and other interested parties to understand the principles and science behind the risk assessment steps.

Three such consultations have already been convened. Two of these, one in July 2000 and one in May 2001 have dealt with the risk assessment of *Salmonella* spp. in broilers, *Salmonella* Enteritidis in eggs and *Listeria monocytogenes* in ready-to-eat foods. These assessments are currently near completion. In July 2001 another expert consultation addressed risk assessment of *Campylobacter* spp. in broiler chickens, and *Vibrio* spp. in seafood. Work on these will continue for another year. The work plan and priorities

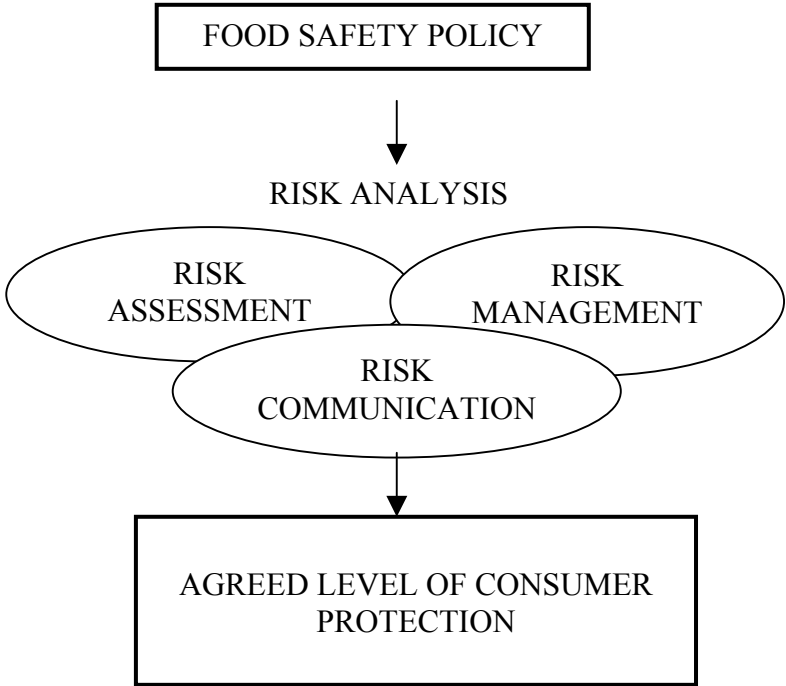
programme for work on MRA are established by FAO and WHO, taking into consideration the needs of the CCFH, as well as the member countries.

3. MRA, international food safety standards and future trends

3.1 Setting of current international standards

Based on the SPS Agreement, food safety standards need to be based on sound science and risk assessment. Figure 1 shows how these standards are set. The starting point is the relevant food safety policy. By using risk analysis, this policy is transformed into food safety objectives, which equate with an agreed level of consumer protection.

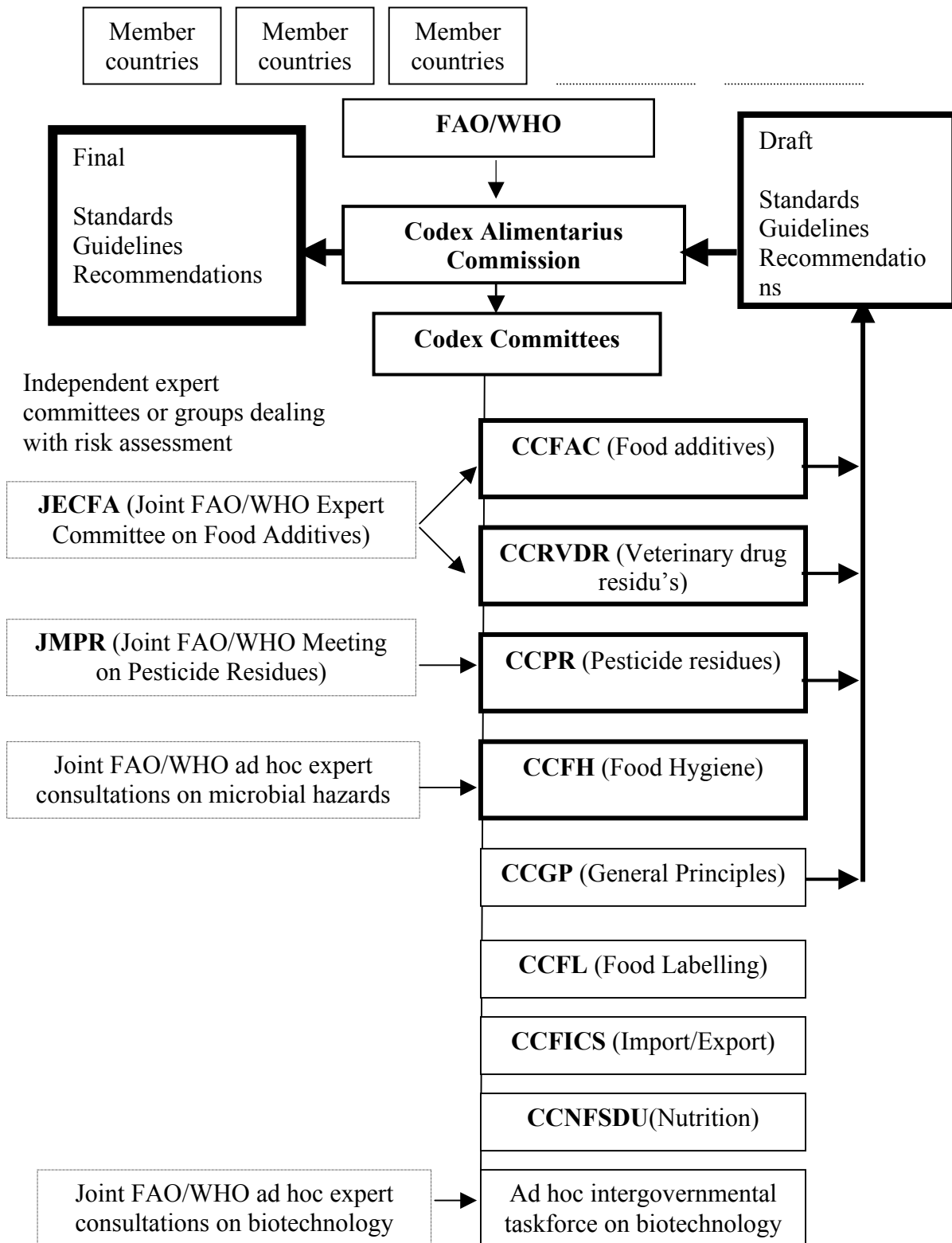
Figure 1. The use of risk analysis to convert a the food safety policy into food safety objectives



Currently, FAO and WHO are the organisations concerned with food safety at the international level. As far as international food safety standards are concerned, these are established under the Joint FAO/WHO Food Standards Programme by the CAC. This organisation has delegated the development of standards, guidelines and other recommendations to its subsidiary bodies, which are guided by the CAC. Normally, the general subject Codex committees (described as ‘horizontal’ Codex committees) are more routinely involved in risk management. These include the Codex committees on Food Additives and Contaminants, Pesticide Residues, Residues of Veterinary Drugs in Food, Food Hygiene, General Principles, Food Labelling, and Nutrition and Food for Special Dietary Uses. The tasks of these inter-governmental bodies are to prepare draft standards, guidelines and recommendations for consideration by the CAC.

The process of setting international food safety standards is expressed in Figure 2.

Figure 2. Visualisation of the process of setting international food safety standards



Initiating the process of standard setting. The risk analysis procedure is usually initiated by one of the respective Codex committees, when it proposes setting standards for additives, contaminants, microbiological agents etc. This process may also be triggered by direct requests to FAO/WHO from member countries. The initiation of the evaluation procedure serves as the hazard identification step.

Risk assessment. The first step in the process of risk analysis is risk assessment, which is carried out by independent expert committees or groups that advise the respective Codex committees. At present, there are two long-standing expert groups that provide advice to Codex, governments and industry. They are the JECFA and the Joint FAO/WHO Meeting on Pesticide Residues (JMPR). In addition, FAO and WHO convene ad hoc expert consultations, as required, to address specific issues not covered by JECFA or JMPR. In recent years, several expert consultations have been held on microbiological hazards in food, the risk assessment of foods derived from biotechnology and on animal feeding and food safety. Recently, the CAC, at its Twenty-fourth Session, held in Geneva, Switzerland from 2-7 July 2001 requested FAO and WHO to further strengthen scientific support for science-based decision making. FAO and WHO, conscious of the importance of this issue, are currently studying the possibility of harmonising the risk assessment procedures used by the various scientific advisory groups and are looking for ways to improve the quality, quantity and time-lines of scientific advice.

JECFA. The work of the JECFA now includes the evaluation of contaminants, naturally occurring toxicants and residues of veterinary drugs in food. For food additives, the JECFA normally establishes so-called acceptable daily intakes (ADIs) on the basis of available toxicological and other relevant information. Specifications for identity and purity are also developed for food additives, which help to ensure that the product in commerce is of appropriate quality, can be manufactured consistently, and is equivalent to the material that was subjected to toxicological testing. For contaminants and naturally occurring toxicants, levels corresponding to 'tolerable' intakes, such as the provisional maximum tolerable daily intake (PMTDI) or provisional tolerable weekly intake (PTWI) are normally established when there is an identifiable no-observed effect level. If such a level cannot be identified, the Committee may provide other advice depending on the circumstances. In the case of veterinary drugs, data on good practice are evaluated and corresponding maximal residue levels (MRLs) in animal tissues, milk or eggs are recommended. Such MRLs are intended to provide assurance that when the drug has been used properly, the intake of any residues of the drug in food is unlikely to exceed the acceptable daily intake (ADI).

JMPR. The JMPR comprises the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and in the Environment and the WHO Core Assessment Group. The JMPR carries out toxicological evaluation of pesticide residues, normally resulting in an estimate of the ADI. In addition, the JMPR proposes MRLs for individual pesticides in or on specific commodities. These MRLs are primarily based on the residue levels estimated in supervised field trials, when the pesticide is used according to Good Agricultural Practices (GAP). In cases where initial estimates indicate that the ADI may be exceeded, more refined intake calculations are performed, using national food consumption data and information from pesticide residue monitoring programmes.

Both the JECFA and JMPR establish chemical safety standards that are based on a review of toxicological studies in the most sensitive test-animal species. They allow for an adequate level of safety, use risk assessment procedures, consider use and consumption patterns and define specifications for the identity and purity of food grade chemicals to be used.

For microbiological hazards, there is currently no JECFA or JMPR-like body. For food safety risk assessment activities, ad hoc expert consultations are set up and independent and appropriately qualified experts are invited. A procedure for this process, adopted and in use since 2000, enhances the principles of transparency, equal opportunity, excellence and independence, and seeks to harmonise the working procedures between different expert bodies and between FAO and WHO. Briefly, the procedure involves the following steps:

1. An open call for experts is made 6 months prior to each expert meeting.
2. Review of candidates by a four-member selection panel.
3. Completion of a “Declaration of Interests” form, indicating institutional affiliation by candidates.
4. Secretariat selects appropriate individuals.
5. Secretariat notifies Governments of the selected experts to obtain their consent.
6. Secretariat invites the experts.

Risk management. The risk management activities are carried out by the respective Codex committees, comprising participants from all member countries, including representatives of industry, consumers and governmental bodies. These representatives carry out the risk management part of the standard-setting procedure. Draft standards, guidelines and recommendations are elaborated via an eight-step process (or in some cases a five-step, accelerated process) by the Committees. The final decision regarding their adoption is made by the CAC.

3.2 International criteria: Future trends

The generic frameworks of current food safety systems used for chemicals and microbiological agents show some similarities, but also important differences. The frameworks for both entities are presented in Figure 3, which is based on the paper given by Hathaway at the IAFP congress, held in Minneapolis, 2001 (Hathaway, 2001).

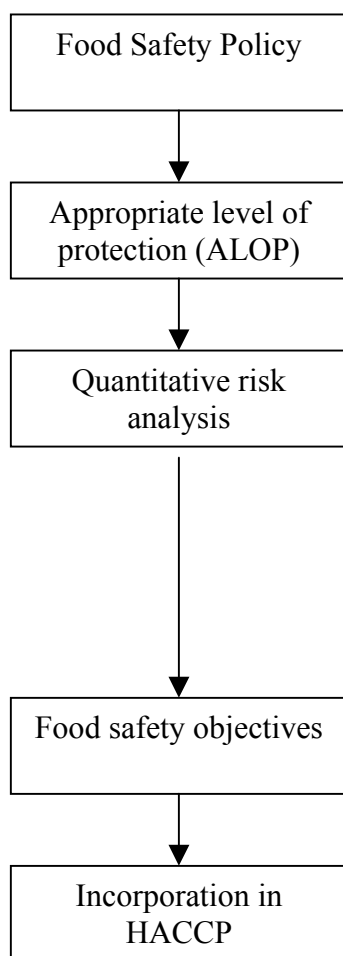
For the setting of international criteria for chemicals, an international food safety policy has been developed. The policy comprises certain general rules. Examples of these are that carcinogens should be absent from food and the aim should be to follow the ALARA principle, which means that, for extraneous chemicals, levels ‘As Low As Reasonably Achievable’ are required. Also, an appropriate level of protection has been agreed. For most chemicals, levels below the no-effect level, including an uncertainty factor, are considered to provide an appropriate level of protection (ALOP). The risk assessment process is primarily directed to assessing the characteristics of potentially hazardous agents and exposure assessment. The risk management process, that is carried out by the relevant Codex Commission, results in the final food safety objectives. These must be incorporated as the required process, product and storage criteria in the HACCP system.

With the passage of time, the system used for chemicals has proved to be very effective in preventing foodborne illness from this source. Actually, with some exceptions, chemical

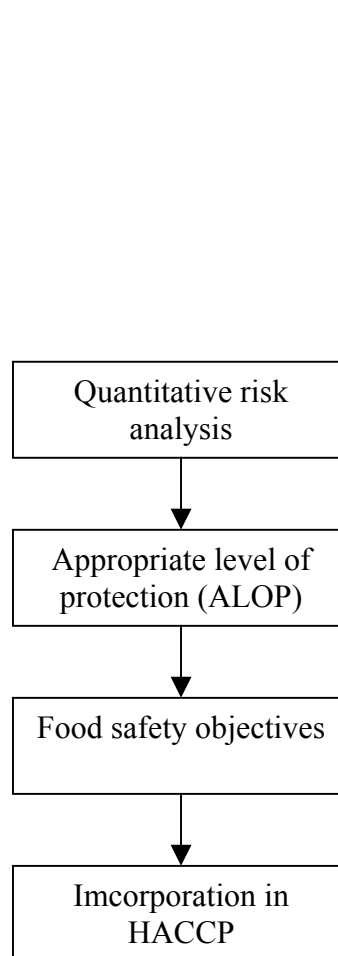
contaminants and residues do not cause overt health problems, and in that respect they are quite different from microbiological agents. Almost all reported foodborne illness is caused by pathogenic organisms present in food.

Figure 3. Generic framework of current food safety systems as developed for chemicals (additives, pesticides, etc.) and for microbiological agents (Based on Hathaway, 2001).

A) CHEMICALS



B) MICROBIOLOGICAL AGENTS



As far as microbiological agents are concerned, there is, at present, no food safety policy associated with the setting of international criteria. Also, unlike chemicals, there is no concept of any levels of product contamination with specific pathogens that would provide an ALOP. The approach to microbiological food safety can be summarised as follows. The system begins with a quantitative risk analysis. Depending on the outcome, appropriate levels of protection are agreed and food safety objectives set. These objectives then need to be reflected in process, product and storage criteria for incorporation into the HACCP system.

There is some debate about whether a unified approach should be developed for both chemicals and microbiological agents, and essential differences in the risks that they pose to

human health need to be understood and taken into account. Other important factors are given in the following:

- *Stability*. While concentrations of most chemicals remain relatively stable in foods during storage, microbial contaminants may die-off or even multiply, depending on the conditions.
- *Behaviour*. The storage behaviour of microorganisms in foods is affected by various intrinsic and extrinsic factors, and can vary considerably from food to food and from one organism to another.
- *Origin*. Chemical contamination of foods with residues of veterinary drugs, pesticides, etc comes from extraneous sources, but many microorganisms occur naturally, especially in raw foods, and their presence cannot be avoided.
- *End-product criteria*. Although clearly useful for chemicals, such criteria are of less value for microorganisms. This is largely due to changes in microbial counts with time and the difficulty of detecting low numbers of specific pathogens, which, if present, are often distributed unevenly in the food. Therefore, a negative result is no guarantee that the target organism is entirely absent from the test batch.
- *Exposure assessment*. Because of the above-mentioned changes in microbial populations during storage, the value of any counts obtained for the purposes of exposure assessment will depend upon the timing of the tests and the subsequent storage history of the food.
- *Assessment of dose-response relationship*. The necessary information for microbial pathogens cannot be obtained from animal experiments and must be taken from feeding trials involving human volunteers or be based on count data from foods associated with specific and well-documented outbreaks.

It is clear that the risks to consumers from chemicals in foods are very different from those presented by microbial pathogens, and a unified approach to their regulation may not be feasible, as far as the setting of criteria for the end-product is concerned. The problem is compounded by the practical difficulties that arise when considering the dynamic nature of microbial populations in foods and the uncertainties surrounding the detection of pathogens. Therefore, the systems used in each case to ensure the required level of food safety are likely to remain separate for the foreseeable future.

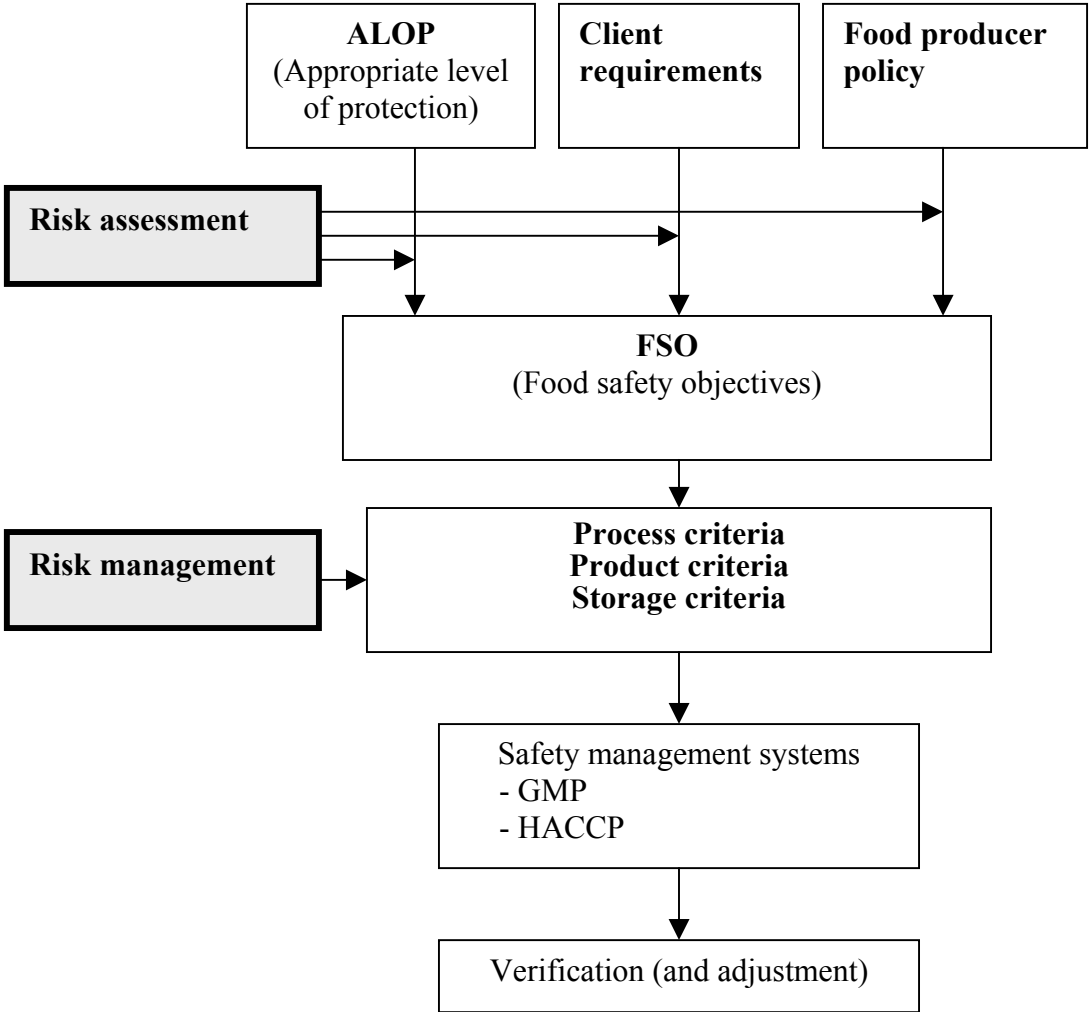
4. Present and future uses of MRA in the Food Industry

4.1 Trends in food safety control

Traditionally, food safety is assessed retrospectively through microbiological testing of randomly selected food samples. This is done by both the food producer and the appropriate regulatory body. The approach may confirm that the food meets certain statutory criteria at the point of sampling, but takes no account of the likely changes in microbial populations during subsequent handling and storage of the product up to the point of consumption. In practice, there is usually no information on whether such control criteria are effective in protecting consumers. Because of these shortcomings, food safety control is increasingly dependent on a more prospective approach, involving the application of GMP and HACCP principles. For this purpose, the use of predictive microbiology has proved to be valuable, as it was previously in developing processes for e.g. heat inactivation of microorganisms and their spores. Recent progress in predictive modelling has facilitated exposure assessment at

each stage of the food chain and has permitted the introduction of risk analysis, which has provided a new milestone in the production of safe food. Thus, acceptably safe food can be produced almost entirely in a prospective and predictable manner, and it is possible to predict that any necessary criteria can be met at the time the food is consumed. The modern approach to safe food production, including the role of GMP, HACCP and risk assessment, is shown schematically in Fig. 4. The first step requires a quantitative risk assessment to identify the hazards.

Figure 4. Schematic presentation of the manner in which microbiologically safe food is produced and the role of risk assessment and risk management. For further explanation see text.



These are then characterised, mostly in terms of dose-response relationships and the severity of the illness caused, followed by exposure assessment and risk characterisation. Finally, risk management requirements are established, using Codex Alimentarius standards, guidelines and recommendations. These involve all interested parties, such as food producers, regulatory authorities, consumer organisations and scientists (the so-called stakeholders). However, any resultant microbiological standards are of limited value, for the reasons discussed previously, and only useful for microbiologically stable food products. Therefore, participants at the IAFP congress in Minneapolis, USA in August 2001, including members of

the ICMSF, proposed a change from control based on food standards to a system involving ALOP at the time of consumption of the food.

An ALOP results from the outcome of a risk assessment, taking account of the costs involved in any control action. Such an analysis is made by the stakeholders, with the knowledge that reducing the risk of a hazard occurring will increase the food production cost, but is hardly likely to reduce the risk to zero. The nature of the ALOP depends very much on the severity of the hazard and the type of food in question. For canned foods that are purchased by large numbers of consumers, the ALOP for toxigenic *Clostridium botulinum* implies that the occurrence of botulism is reduced to a negligible level. In the canning of low-acid foods, it is generally agreed that the ALOP requires the use of a process giving a (theoretical) 10^{11} - 10^{12} -fold reduction in the level of *Clostridium botulinum*.

For freshly cut vegetables that are eaten raw, the ALOP may require a 50% reduction in foodborne disease over a 10 year period. A similar kind of target has been set in the USA by the FDA for raw poultry meat (Buchanan, 2001). Clearly the targets must be expressed in terms of food safety objectives. In relation to poultry meat, a 50% reduction in disease over 10 years can only follow from a corresponding decrease in pathogen contamination of poultry carcasses. The relevant calculation can now be made from a proper risk assessment. From the producer's viewpoint, the meeting of Food safety objectives is just one consideration. Account must also be taken of any specific customer (retailer) requirements as well as the producer's own profitability.

In producing safe food, there are various aspects, which can be grouped in three main categories:

1. The type of process used, which may include heat treatment, irradiation, high-pressure technology etc.
2. Product composition, including addition of e.g. salt, acids or other preservatives.
3. Storage conditions, involving storage temperature and time, gas packaging etc.

Effective management of these aspects allows all food safety requirements to be met. In doing so, it is necessary to define criteria for process performance, product composition and storage conditions. The setting of the criteria is the task of the risk manager, and use of the HACCP concept is the managerial tool to ensure that the criteria will be met in practice. Finally, a verification step is needed to demonstrate that the ALOP, the customer requirements and the producer's own objectives are being met. If, for any reason, it is impossible to meet the ALOP, then production of the food in question must cease.

4.2 Some examples

4.2.1 Setting storage criteria for pasteurised milk.

The presence of *Bacillus cereus* in pasteurised milk should be considered hazardous, because the organism is potentially pathogenic and can multiply in this milieu. The organism is also associated with foodborne illness resulting from the consumption of dairy products.

In most European countries, a limit value of 10^4 organisms per ml or gram at the time of consumption has been set for dairy products and other foods. Some countries, including The Netherlands, accept the presence of 10^5 per ml in milk, and to meet this limit, the storage criteria for pasteurised milk are 7°C for a maximum of 7 days. Human exposure to *Bac. cereus* from milk consumption was studied by Notermans et al. (1997). Exposure was assessed by (i) enquiring about storage conditions (temperature and time) for pasteurised milk that were used by households in The Netherlands and (ii) carrying out storage trials at 6-12°C. The temperatures studied were those observed in a survey of Dutch domestic refrigerators. The probability of exposure to different doses of *Bac. cereus* is given in Table 5.

Table 5. Exposure to *Bacillus cereus* after consumption of pasteurised milk based on model experiments of Notermans et al. (1997)

Exposure dose (organisms/ml)	Probability (%)
> 10^0	99
> 10^2	21
> 10^3	14
> 10^4	11
> 10^5	7
> 10^6	4
> 10^7	<1

The results demonstrated that 7% of the milk contained $>10^5$ *Bac. cereus* per ml at the time of consumption. It was also shown that storing milk according to the producer's recommendations would prevent the limit value of 10^5 per ml from being exceeded.

Risk management options. It is clear that managerial action is required to ensure that the official criterion is met. In order to take such action, it is necessary to assess the predominant factors that determine the final level of *Bac. cereus* when the milk is consumed. These are:

- The initial level of contamination with the organism (N_0), which is influenced by factors such as the grazing period for the cows and control of hygiene during milking.
- The storage time (t) for the pasteurised milk.
- The storage temperature (T) of the milk.

Zwietering et al. (1996) derived an equation for calculating the effects of each of the above variables on the numbers of *B. cereus* finally present (N).

$$N = N_0 \cdot e^{0.013 \cdot T^2 \cdot t}$$

From the equation, it can be observed that storage temperature has the largest effect on the level of *Bac. cereus* at the time the milk is consumed. This is followed by storage time, while initial count has only a minor effect. The effects are illustrated by the data presented in Table 6.

Table 6. Storage times for pasteurised milk giving a final count of *Bacillus cereus* of 10⁵/ ml: Effects of initial number and storage temperatures (Notermans et al, 1997).

Initial number per ml	Storage temperature (°C)			
	6	8	10	12
0.001	13.4 ^{*)}	7.6	4.8	3.4
0.01	11.3	6.4	4.1	2.8
0.1	9.2	5.2	3.3	2.3
1	7.0	4.0	2.5	1.8
10	4.9	2.8	1.8	1.2

^{*)} Storage time in days

Selection of new control options. The simplest option for the milk producer would be to do nothing, since the prescribed storage conditions are quite adequate on the label. However, the situation is different if consumer complaints start to increase and the producer loses business. In this case, a lower storage temperature could be recommended on the label, although it is by no means certain that consumers would respond by reducing the temperature in their refrigerators. In addition, many countries have stipulated temperatures for storing chilled foods, e.g. 7°C in the Netherlands, and any decrease would involve negotiations with trading authorities, retailers and consumer bodies. The last option would be to reduce the maximum storage time, but this would raise other considerations. Retailers, for example, may well favour such a step, for the simple reason that consumers would need to purchase milk more frequently. While reducing the temperature would possibly be more costly to the retailer, a shorter storage time would necessitate more frequent deliveries and therefore be an additional cost to the supplier.

Because of progress in predictive modelling, the risk assessor is able to determine the effect on product safety of different storage conditions. It is, however, the risk manager who has to make the final decision on the action to be taken, and this involves consideration of all the relevant aspects of the problem.

4.2.2 From targets to HACCP criteria

In many countries, poultry meat products contribute significantly to foodborne disease, especially that caused by *Salmonella* and *Campylobacter* spp. Although various attempts have been made to improve the situation, little progress has been made until recently. One of the reasons may be the continuing deadlock in accepting responsibility. Consumers expect pathogen-free products, which cannot be achieved at the present time, while producers refer to the unhygienic practices of consumers, when food is prepared in the kitchen. In order to change this situation in the USA, the FDA has set a target, whereby foodborne disease from poultry meat will be reduced by 50% over a ten-year period (FDA, 2001) and producers are held responsible for meeting the target.

For operational purposes the target, which is an ALOP, needs to be translated into appropriate process, product and storage criteria. To set the criteria, it is necessary to calculate the requisite reduction in contamination of poultry meat with the key pathogens (food safety objectives). The following steps are required:

1. Assessment of the prevalence of *Salmonella* and *Campylobacter* spp. in commercial broiler flocks.
2. Quantitative assessment of product contamination with the pathogens at the end of processing.
3. Determination of the effect of storage on pathogen contamination.
4. Assessment of the effects of food preparation by consumers on the survival and spread of the pathogens.
5. Collection of consumption data.

The information thus provided will allow an assessment to be made of human exposure to the pathogens at the time the food is consumed. Existing dose-response relationships can be used to determine the likely number of disease incidents or the probability of disease. These figures now need to be reduced by 50% and the new target for exposure can be determined again from the dose-response relationships.

There are several ways in which the new food safety objectives can be met. One approach is to set process performance criteria, which might include low environmental temperature, minimum processing time, spraying carcasses with lactic acid, washing in chlorinated water etc. Also, product storage conditions (low temperature, short time) may be important to minimise any risk of growth of pathogens. The processing procedures and the conditions of processing and storage provide the CCPs in the HACCP system. If information is available, critical limits can be based on published data, although this is not always possible for specific parts of the process or storage conditions. Until more sophisticated models have been developed, the necessary calculations must be based on simple D-values and modelling of growth parameters.

5. Current issues in microbiological risk assessment

As with all risk assessment procedures, MRA comprises hazard identification, hazard characterisation, exposure assessment and risk characterisation as the four basic elements. It is a relatively new discipline in relation to the production of microbiologically safe food. However, the principles embodied in the approach have been applied for many years, especially to heat processes developed for low-acid canned foods and treatment of milk in the 1930s and 1940s respectively. The resultant heating regimes have proved to be very successful in controlling any foodborne diseases that might be associated with these sources.

Although MRA was first introduced as a food safety measure in 1995, its use has been limited and the approach has yet to lead to internationally recognised microbiological criteria. It may take longer than anticipated for the concept to be universally accepted and applied, but initiatives taken by the WHO and FAO to organise meetings of experts (see Section 2.6 of this chapter) could help to stimulate interest. It should be recognised, however, that the application of MRA in the production of safe food will be hampered by the present lack of any comprehensive, microbiological food safety policy. The problem is compounded by the large diversity of available food products that vary from fully processed up to almost un-processed ready to eat products. Within these categories, there are differences in processing methods, product composition and storage conditions. In addition, microbial contamination may be introduced into the food chain, sometimes from the raw materials used in product manufacture. In other instances such contamination may come from organisms that are

endemic in the processing environment or through human handling of the food etc. Despite the use of various processes for reducing the microbial load on food, consumer safety cannot always be guaranteed because of the recontamination problem.

As well as the above-mentioned product diversity, there are significant differences between products in the types of micro-organisms that may be present. Among the variety of possible foodborne pathogens are rickettsiae, viruses, bacteria, moulds and parasites. Each of these groups contains organisms with particular growth characteristics, ecological behaviour and disease potential. With so much variation between products and in the nature of the contaminants present, it is hardly surprising that separate risk assessments are not really feasible. This is especially so if end-product criteria based on MRA are required in each case. Such an approach has been attempted recently by the ICMSF, but without real success. For example, in relation to potential growth of *Listeria monocytogenes* in food and the varying sensitivity to this pathogen among different human groups, the ICMSF proposed 15 separate categories of food, each with its own food safety objective (FSO).

Among other weak points in current attempts to use MRA are difficulties in (i) exposure assessment, (ii) assessment of dose-response relationships and, consequently, (iii) the uncertain outcome of risk characterisation. Another aspect to be considered is human perception, which has no direct relationship to health problems, but carries significant implications for consumer confidence in the safety of the food supply. Finally, it should be noted that mistakes are sometimes made in attributing human illnesses to the consumption of contaminated food and a misleading impression may result.

Exposure assessment. Microbiological models are an important tool for this exercise. Suitable models are necessary because it is impracticable to test individual food products for this purpose (see Section 2.3 of this chapter). The value of end-product testing is mainly in relation to verification procedures, which are discussed below.

For the purposes of exposure assessment, the Monte Carlo type of model is particularly relevant and is based on the distributions of all appropriate variables in the food production process. These will include product composition and storage conditions, consumption habits etc. The approach involves taking random values for each of the distributions to assess the final exposure distribution. A weakness is that the distributions of variables must be independent of each other and often this is not the case. For example, storage time and temperature for pasteurised products are usually inter-dependent. Nevertheless, the Monte Carlo approach provides much more realistic data, when compared with a worst-case scenario, as used in the past. Furthermore, it provides information that takes account of the uncertainty or variability in human exposure to micro-organisms. Experimental data on human exposure to pathogenic organisms via beef hamburgers (Cassin et al., 1998) and *Salmonella* Enteritidis from pasteurised liquid egg (Whiting and Buchanan, 1997) show that exposure levels can vary widely, although no allowance was made for error.

Dose-response relationship. In risk assessment, much attention is given to dose-response relationships, which are considered essential in risk assessments for toxins, food additives, drug residues etc. After MRA became a legal requirement in 1995, attention was also focussed on micro-organisms in this respect. Although it is clear that experimental use of animal models has provided important information on mechanisms of pathogenicity in organisms such as *L. monocytogenes* (Notermans et al., 1998), the data obtained cannot be used to derive dose-response relationships for humans. Instead, such relationships are mainly

based on data from human volunteer studies or the analysis of foodborne disease outbreaks involving micro-organisms. These data show considerable variation, even between the serotypes of *Salmonella* (Kothary and Babu, 2001). Currently, however, reliable information on microbiological dose-response relationships is still very scarce. Among the difficulties, is the fact that challenge studies on volunteers can only be carried out with the less dangerous pathogens and, of course, the volunteers will be only healthy adults. In practice, foodborne infections are commonly seen in the more vulnerable groups within the general population (infants, the elderly, people undergoing treatment with immunosuppressive drugs, people with AIDS). These individuals can comprise about 20% of the whole population.

A further aspect, which must be taken into account, is the physiological condition of the disease agent. This will affect virulence and, in turn, the dose-response relationship (Abee and Wouters, 1999). The situation is complicated by the ability of some micro-organisms to protect themselves against external stress factors that might arise in minimally processed food products (Abee and Wouters, 1999; Hecker and Völker, 1998). Protection may also be afforded against the acid conditions of the stomach, during passage of the contaminated food following ingestion (Abee and Wouters, 2002). On the other hand, virulence may be adversely affected by the nature of the food matrix, within which the organism is contained, so various factors must be considered.

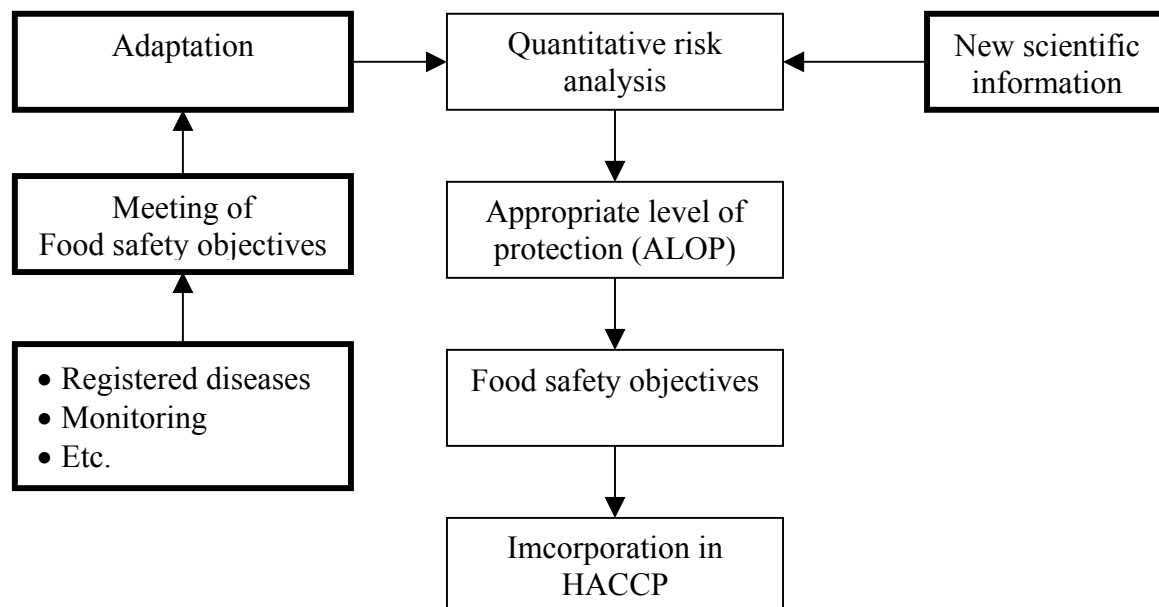
Risk characterisation. This is the outcome of exposure assessment and establishment of the dose-response relationship, taking account of the severity of illness caused by a particular pathogen. However, it suffers from the fact that both exposure assessment and dose-response analysis are not yet clearly established in MRA. Only time will tell whether the present approaches in exposure assessment and dose-response analysis will result in widespread acceptance and application of MRA. There is some debate about the possible use of epidemiological data on microbial foodborne illness as an alternative for the purposes of risk characterisation. Because relevant information is lacking in nutritional risk assessment, use of epidemiological data has become common and is applied successfully. In relation to microbial foodborne illness, data collected in countries such as the USA, UK and The Netherlands could be used to determine, for example, the incidence rate for human salmonellosis caused by egg consumption, eating of poultry meat etc. The uncertainty of the outcome of that kind of calculation is relatively well defined and very much less than that from data based on exposure assessment and dose-response modelling. Also, the use of epidemiological methods, such as case-control and cohort studies, allows the most important risk factors to be identified.

Where to go from here? The introduction of MRS is essential in order to assess the risk and severity of a microbial foodborne disease. For the management of an unacceptable risk, FSOs would need to be formulated. These should not be simply microbiological criteria, such as a specified number of cells of a particular pathogen that can be present in a food at the time of consumption. A better approach is that used recently in the USA, where targets have been established as FSOs for raw poultry and red meat products (Buchanan, 2001). The target for foodborne disease caused by poultry consumption is to reduce the present level by 50% over a period of 10 years (see Section 4.2.2 of this chapter). Setting a target for raw products of this kind is an attractive proposition, but its success depends largely on the availability of an appropriate means of reducing microbial contamination of the product and a reliable system for collecting data on foodborne disease.

Verification. Currently, an important issue in microbiological risk analysis is the process of verification, which is a means of determining whether the analysis, including MRA, has been

carried out correctly and that an acceptable level of protection has been obtained. The process of verification is presented schematically in Figure 5.

Figure 5. The process of verification of microbial risk assessment



Verification comprises several elements: (i) an evaluation to determine whether the risk analysis resulted in FSOs and, when introduced, whether these met the expectations of the stakeholders, ie all those involved in the process and, if not, (ii) adaptation of the FSOs, or (iii) re-evaluation of the MRA. The last step is also relevant when new scientific information becomes available that questions the value of the MRA. Adaptation of FSOs may also be necessary as a result of epidemiological data on the frequency of foodborne diseases, data from microbiological monitoring of the food product or any new information, such as that involving changes in risk factors. This last point illustrates the dynamic nature of the circumstances involved in the production of microbiologically safe food.

6. References

Abee T and Wouters J A (1999), 'Microbial stress response in minimal processing;', *Int. J. Food Microbio.* **50** 65-91.

Abee T and Wouters J A (2000), 'Stress response and food safety' in Symposium Proceedings 'Frontiers in Fermentation and Preservation' Joint meeting Society for Applied Microbiology, UK / The Netherlands Society for Microbiology, Wageningen 2002.

American Public Health Association, Subcommittee on Methods for the Microbiological Examination of Foods, 'Recommended methods for the microbiological examination of foods'. 2nd ed. New York, American Public Health Association Inc, 1966.

Anon (1995), 'Trading into the future', World Trade Organisation, Geneva.

Baird-Parker T (2000), 'The production of microbiological safe and stable food', in Lund B M, Baird-Parker T C and Gould G W, *The Microbiological Safety and Quality of Food*, Gaithersburg, Aspen Publishers, 3- 18.

Ball C O, 'Thermal process time for canned food', *Bull. Natl. Res. Council No. 37 Vol 7, Part 1*. Natl. Res. Council Washington, DC, 1923.

A.W. Barendsz, 1995 'Kwaliteitsmanagement: HACCP de ontbrekende schakel' in the book '*HACCP, a practical manual*', Keesing Noordervliet, ISBN 90 72072 40 5

Bergdoll M S (1989), 'Staphylococcus aureus' in Doyle M P, *Foodborne Bacterial Pathogens*, New York, Marcel Dekker Inc, 464-524.

Bigelow W D, Bohart G S, Richardson, A C and Ball C O, 'Heat penetration in processing canned foods', *Bull. No. 16-L, Res. Labs. Natl. Cannery Assoc.*, Washington DC, 1920.

Brown G L, Colwell D C and Hooper, W L, 'An outbreak of Q fever in Staffordshire. *Journal of Hygiene, Cambridge* 1968 **66** 649-655.

Bryan, F.L. (1992) '*Hazard analysis critical control point evaluations: a guide to identifying hazards and assessing risks associated with food preparation and storage*'. WHO document, 1992.

Buchanan R L et al (2001), 'Moving beyond HACCP – Risk management and food safety objectives', in Symposium abstracts IAFP 88th Annual Meeting, Minneapolis 2001.

CAC/GL30 (1999), '*Principles and Guidelines for the Conduct of Microbiological Risk Assessment*'. Codex Alimentarius Commission. Food and Agriculture Organisation, World health Organization, Rome.

CAC, Committee on Food Hygiene (1991). *Draft principles and applications of the Hazard Analysis Critical Control Point (HACCP) system*. Alinorm 93/13, Appendix VI. Food and Agriculture Organisation, World health Organization, Rome.

CAC, Committee on Food Hygiene (1997), *Hazard Analysis Critical Control Point (HACCP) and guidelines for its application*. Alinorm 97/13. Food and Agriculture Organization, World health Organization, Rome.

Chung K-T, Stevens S F and Feris D H, 'A chronology of events and pioneers of microbiology' *SIM News*, 1995 **45** 3-13.

D'Aoust J-Y (1989), 'Salmonella' in Doyle M P, *Foodborne Bacterial Pathogens*, New York, Marcel Dekker, Inc 328-446.

Department of Health, Education and Welfare, Proceedings 'National Conference on Food Protection', Washington, D C US Governmental Printing Office, 1972.

Derrick E H, "'Q" fever, A new fever entity: Clinical features, diagnosis, and laboratory investigation. *Med J Australia*, 1937 **2** 281-299.

Dobell C (1960), Antony van Leeuwenhoek and his "Little Animals.", New York, Dover Publications.

Doyle M P (1989), *Foodborne Bacterial Pathogens*, New York, Marcel Dekker, Inc 328-446.

Enright J B, Sadler, W W and Thomas R C, 'Thermal inactivation of *Coxiella burnetii* and its relation to pasteurisation of milk', Public Health Service Publication No. 517. United States Government Printing Office, Washington, D C, 1957

Enright J B, Sadler W W and Thomas, R.C. 'Observations on the thermal inactivation of the organism of Q fever in milk', *J Milk Food Technol*, 1956 **10** 313-318.

Esty J R and Meyer K F, 'The heat resistance of spores of *Bacillus botulinus* and allied anaerobes', *XI J Inf Dis*, 1922 **31** 650-663.

Farber J M and Peterkin P I (2000) 'Listeria monocytogenes', in Lund B M, Baird-Parker T C and Gould G W, *The Microbiological Safety and Quality of Food*. Gaithersburg, Aspen Publishers, Inc, 1178-1232

FAO/WHO (1995), *Application of risk analysis to food standards issues*, Report of a joint FAO/WHO Expert Consultation, World Health Organisation, Geneva, Switzerland 1995.

FAO/WHO (1997), *Risk management and food safety*, Report of a joint FAO/WHO Expert Consultation, Rome, Italy, 1997.

FAO/WHO (1998), *The application of risk communication to food standards and safety matters*, Report of a joint FAO/WHO Expert Consultation, Rome, Italy, 1998.

FAO/WHO (2000a), *Risk assessment of microbiological hazards in foods*, Report of a joint FAO/WHO Expert Consultation, Rome, Italy 2000.

FAO/WHO (2000b), *The interaction between assessors and managers of microbiological hazards in food*, Report of a joint WHO Expert Consultation, Kiel, Germany 2000.

FDA (1973), 'Acidified foods and low acid foods in hermetically sealed containers' in Code of US Federal Regulations, Title 21, 1 Parts 113 and 114 (renumbered since 1973) FDA Washington DC.

Granum Per Einer (1997), 'Bacillus cereus' in Doyle M P, Beuchat L R and Montville T J, *Food Microbiology: Fundamentals and Frontiers*, Washington, ASM Press, 327-336.

Hartman P A (1997), 'The evolution of food microbiology', in Doyle M P, Beuchat L R and Montville T J, *Food microbiology: Fundamentals and frontiers*, Washington, ASM Press, 3-13.

Hathaway S C (2001), 'An International Perspective on Food Safety Objectives' – Risk management and food safety objectives', in Symposium abstracts IAFP 88th Annual Meeting, Minneapolis 2001.

Hauschild A H W (1982), 'Assessment of botulism hazards from cured meat products', *Food Technol*, **36** 95-104.

Hecker M and Völker U (1998) 'Non-specific, general and multiple stress resistances of growth-restricted *Bacillus subtilis* cells by the expression of the σ^B regulon' *Mol Microbiol* **29** 1129-1136.

Hutt P B and Hutt P B II, 'A history of government regulation of adulteration and misbranding of food', *Food Drug Cosm Law* 1984 **39** 2-73.

IAMFES (International Association of Milk, Foods and Environmental Sanitarians, Inc.) (1991), *Procedures to implement the hazard analysis critical control point system*. IAMFES document 502.

ICMSF, (The International Commission on Microbiological Specifications of Foods) (1988), *Micro-organisms in foods. Application of the hazard analysis critical control point (HACCP) system to ensure microbiological safety and quality*. Oxford, Blackwell Scientific Publications

ICMSF, (The International Commission on Microbiological Specifications of Foods) (1974), *Micro-organisms in foods 2. Sampling for microbiological analysis: Principles and specific applications*, Toronto, University of Toronto Press

Kampelmacher E H, *Since Eve ate apples*, Inaugural address 11 November, Wageningen University, 1971.

Kotharyl M H and Babu U S (2001) 'Infective dose in volunteers: a review' *J. Food Safety* **21** 49-73.

Lund B M and Peck M W (2000) 'Clostridium botulinum', in Lund B M, Baird-Parker T C and Gould G W, *The Microbiological Safety and Quality of Food*. Gaithersburg, Aspen Publishers, Inc, 1057-1109.

Maurin M and Raoult D, 'Q Fever'. *Clinical Microbiology Reviews*, 1999 **12** 518-553.

McClane B A (1997), 'Clostridium perfringens' in Doyle M P, Beuchat L R and Montville T J, *Food Microbiology Fundamentals and Frontiers*, Washington, ASM Press, 305-326.

- Monod J, 'The growth of bacterial cultures' *Ann. Rev. Microbiol*, 1949 **3** 371-394.
- Monod J, 'La technique de Culture continue. Théorie et application' *Ann. Inst. Pasteur*, 1950 **79** 390-407.
- Mossel D A A , *Microbiology of Foods, Utrecht*, The University of Utrecht, 1982.
- Notermans S, Gallhoff G, Zwietering M H, and Mead G C, 'Identification of critical control points in the HACCP system with a quantitative effect on the safety of food products' *Food Microbiology* 1995 **12** 93-98.
- Notermans S, Dufrenne J, Teunis P, Beumer R, te Giffel, M and Peeters Weem, P (1997), 'A risk assessment study of *Bacillus cereus* present in pasteurised milk. *Food Microbiol*, **30** 157-173.
- Notermans S, Dufrenne J, Teunis, P. and Chackraborty T, 'Studies on the risk assessment of *Listeria monocytogenes*' *J. Food Protection*, 1998 **61** 244-248.
- Pflug I J and Gould G W (2000), 'Heat treatment' in Lund B M, Baird-Parker T C and Gould G W, *The Microbiological Safety and Quality of Food*. Gaithersburg, Aspen Publishers, Inc, 37-63.
- Ratkowsky D A, 'Principles of nonlinear regression modelling' *J. Gen. Microbiol*, 1993 **12** 245-249.
- Rocourt J and Cossart P (1997), '*Listeria monocytogenes*' in Doyle M P, Beuchat L R and Montville T J, *Food Microbiology Fundamentals and Frontiers*, Washington, ASM Press, 337-352.
- Rose J B and Gerba C P (1991), 'Use of risk assessment for development of microbial standards' *Water Sci. Technol*, **24**, 29-38
- Ross T, and McMeekin T A, 'Predictive microbiology' *Int. Journal of Food Protection* 1993 **23** 241-264.
- Spencer R and Baines C R (1964), 'The effect of temperature on the spoilage of wet fish. I. Storage at constant temperatures between 1 °C and 25 °C' *Food Technology*, **18** 769-772.
- Steinkraus K H, *Handbook of Indigenous Fermented Foods*, New York, Marcel Decker Inc., 1996.
- Tannahill R (1979), *Food in history*, New York, Stein and Day Publishers.
- Toussaint-Samat M, (1992), *History of food*, Cambridge, Blackwell publishers

United States Food and Drug Administration, Division of Microbiology, '*Bacteriological analytical manual*', 3rd ed. Washington DC, 1972.

Untermann F, Jakob P and Stephan R, (1996), '35 Jahre HACCP-System. Von NASA-Konzept bis zu den Definitionen des Codex Alimentarius', *Fleischwirtschaft* **76** 589-594.

Van Ermengem E (1896), 'Ueber einem neuen anaeroben Bacillus und seine Beziehungen zum Botulismus', *Z Hyg Infectiouskrankh*, **26**, 1-56. English translation (1917), *Rev Infect Dis* **1** 701-719

Wendorf F R, Schild R, El Hadidi N, Close A E, Kobusiewicz H, Wieckowska H, Issawi B and Haas H, (1979) 'Use of barley in the Egyptian late paleolithic', *Science* **205** 1341-1348.

Whiting R C and Buchanan R L (1997), 'Predictive Modelling' in Doyle M P, Beuchat L R and Montville T J, *Food Microbiology Fundamentals and Frontiers*, Washington, ASM Press, 728-739.

Zwietering M, Notermans S. and de Wit J (1996), 'The application of of predictive microbiology to estimate the number of *Bacillus cereus* in pasteurised milk at the point of consumption', *Int J Food Microbiol*, **30** 55-70.