=Special Lecture=

Microbiological Risk Assessment as the Basis for Food-safety Criteria

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When properly designed, a microbiological risk assessment (MRA) is an objective and systematic evaluation of existing scientific knowledge to help risk managers making an informed decision about how to handle food safety issues. Together with epidemiological based information and economic analysis it can provide the best information for control measures. One of these control measures concerns setting food safety criteria. In this document some historical backgrounds are presented how food safety management has been evaluated in course of time from mythology, via prohibition and precautionary principles to a science based approach. MRA-tools are described and examples of risk assessment approaches are presented as a basis for setting microbiological criteria.

The concept of food safety has its origins in Greek mythology. A key figure was Asclepius, the god of medicine, who was associated with good health and the cure of disease. The best-known of his children, Hygeia, focussed on the healing power of cleanliness and she was the one to develop the idea of hygiene, on which the microbiological safety of food depends.

However, the gods of Ancient Greece were not the only ones to deal with food-safety issues. In other parts of the world, various religious taboos emerged to protect human health and these prohibited the eating of foods such as pork, which is a requirement of both the Jewish and Muslim religions⁹⁾. Other taboos were concerned with more general principles of food hygiene. In India, for example, religious laws do not allow the consumption of foods that are regarded as 'unclean'. These include meat cut with a sword or sniffed by a dog or cat, or meat obtained from carnivorous animals⁹⁾.

Thus, the first rules were established thousands of years ago, long before there was any knowledge of microorganisms. Such religious laws would have been the only ones in existence. Control measures introduced through civil law appeared much later.

The 'Prohibition' Principle

As their trade in food increased, the early Romans began to pay more attention to spoil-

age and a new rule emerged. It was prohibited to sell spoiled food of any kind. Thus, the aedilis, a kind of religious official, inspected and controlled food markets and confiscated any food that had become spoiled. Over the last 2000 years, the 'prohibition' principle has been widely used in many countries to protect consumers from food that is spoiled or likely to contain dangerous levels of disease agents. Let us consider two specific examples with important implications for food safety.

The first relates to foods containing animal blood and especially sausages¹⁾. A ban on their consumption was introduced during the ninth century by Emperor Leo VI of Byzantium, because he was aware of the high incidence of food poisoning associated with these foods. The law applied particularly to blood sausages and carried a high penalty, if disregarded. It was stated that '*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 scourged and disgracefully shaved, he shall be exiled for life*'¹⁾. From the data available and current knowledge, it is clear that the disease in question was actually botulism.

The second example of the 'prohibition' approach has nothing to do with microbes but is much more relevant to Japan. Here, a dish known as 'fugu' is, historically, one of the most acclaimed and sought-after forms of seafood.

Table 1. Process performance criteria for low acid canned food products⁴⁾

Heat performance criteria for *Clostridium botulinum* in low-acid canned foods are based on:

- the response to heating of the most heat-resistant spores found among strains of *Clostridium botulinum*; and
 - a reduction in the spore population by a factor of 10^{11} – 10^{12} to ensure the desired level of product safety.
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Despite this, there have been many deaths from eating the dish and the problem continues to occur. As long ago as 1550, the Emperor introduced a ban on fugu consumption after a group of soldiers had died. However, the ban was abolished in 1888 when the Japanese Prime Minister tasted a small sample of the dish and suffered no ill effects.

The disease associated with fugu is known as blowfish or pufferfish poisoning. It is due to the neurotoxic effects of the tetrodotoxin, which occurs in various species of pufferfish⁶⁾.

The 'precautionary' principle

Another approach to food-safety control has been the use of the 'precautionary' principle. Despite recent advances in food science, this principle is still relevant in some cases, although its use tends to be restricted to situations involving the more vulnerable groups in society and consumption of foods for which absolute safety cannot be guaranteed. For example, elderly people in the USA are advised to avoid the following foods:

- raw or unpasteurised milk or cheese;
- soft cheeses, such as feta, brie, camembert or Mexican-style cheese;
- raw or lightly-cooked eggs or egg products;
- raw meat or poultry;
- raw sprouts such as alfalfa, clover or radish; and
- unpasteurised or untreated fruit or vegetable juice.

Food-safety advice is also given in some countries to pregnant women, who are warned against eating pâtés and certain soft cheeses, because of the risk of contracting listeriosis. Similarly, parents may be advised to avoid giving honey to infants below one year of age, because honey can contain viable spores of *Clostridium botulinum* and hence there is a risk of botulism⁵⁾. Yet again, the precautionary principle has been applied in relation to bovine spongiform encephalopathy, or 'mad cow disease', especially in risk management. In this

case, there has been a lack of conclusive scientific evidence, but the possibility of litigation means that extreme caution can be justified.

Establishing Process Criteria

Actually, it was already recognised at the beginning of the twentieth century that protection of the public against foodborne hazards required proper control of the heat treatments being used commercially in food production. This was first achieved around 1920 for the sterilisation of canned foods, and methods were established to calculate the necessary heating times for foods processed at different temperatures. In fact, commercial sterilisation does not necessarily destroy all the microbes present. Instead, the goal of thermal processing is to reduce the probability that microbes will survive and subsequently grow in the food to an acceptably low level. Thus, we now speak of 'an appropriate level of protection' and the starting point for the concept was the classical work of Esty and Meyer that was published in 1922⁴⁾. These workers derived process performance criteria for destroying spores of proteolytic strains of *Clostridium botulinum* in low-acid, canned foods.

Requirements for the sterilisation process were based on testing the most heat-resistant spores produced by *C. botulinum* and they involved a reduction of the spore population by a factor of 10^{11} to 10^{12} , thereby ensuring the desired level of product safety. Based on these principles, the resultant performance criteria have been applied successfully for many years and have proved to be sound, with an adequate margin of safety (Table 1).

However, it is important to realise that the work of Esty and Meyer⁴⁾ assumed a worst-case scenario and the same was true of later studies by Enright and colleagues on heat pasteurisation of milk. The aim here was to control the pathogen *Coxiella burnetii* and the work led to the development of process performance criteria. In these studies, published in 1956, risk assessment principles were already beginning

Table 2. The new EU microbiological criteria for ready-to-eat prepared fresh fruit and vegetables as described in Commission Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs

Criteria related to food safety:
<ul style="list-style-type: none"> • the absence of <i>Salmonella</i> in 5 samples of 25 grams • the absence of <i>Listeria monocytogenes</i> in 5 samples of 25 grams • a limit of less than 100 <i>Listeria monocytogenes</i> per gram in 5 samples, if the organism is unlikely to multiply.
Criteria related to the process:
<ul style="list-style-type: none"> • the testing of 5 samples for the presence of <i>Escherichia coli</i>, with 3 being <100 per gram and two between 100 and 1000 per gram.

to appear, because Enright and his co-workers³⁾ carried out dose-response and exposure assessments to support their proposals.

Microbiological Examination of Food

Once the German bacteriologist, Robert Koch, had developed a method for isolating and counting microbes in the laboratory, microbiological testing of foods became possible. This allowed the safety and spoilage potential of a food to be determined and, over the last 80 years or more, many different methods have been devised for detecting pathogenic microbes and their toxins. From the very beginning of this period, legal limits were laid down in various countries and were based on the testing of prepared foods for the organisms or toxins of concern. Thus, there was an emphasis on the microbiological examination of food, which continues to this day. There are, however, certain limitations that need to be recognised and they will be considered in the following.

When pathogens are present in foods, they are usually found irregularly and in low numbers. Therefore, attention has also been given to other organisms that are usually present more consistently and in greater numbers, and are indicative of the presence of pathogens. These 'indicator' organisms provide an indirect means of assessing the microbiological safety and quality of food, and their use flourished, especially in the period 1960 to 1980. During that time, many different methods were developed for the enumeration of bacterial indicators, the main objective being to reveal conditions of food handling and processing that could be hazardous to human health. Where the organisms being enumerated are very similar in their characteristics to the pathogens of concern, they are usually termed 'index' organisms and are more likely to be a good predictor of pathogen behaviour and hence food safety.

How the Criteria Have Been Set

As we have seen, criteria set for process control or microbial contamination of food are often based on worst-case situations, requiring, for example, a very large reduction in the presence of the most heat-resistant spores of *Clostridium botulinum*. Where possible, the criteria take account of epidemiological data and reflect the minimum dose that is expected to cause illness. Despite their scientific basis, however, they do not tell us whether the food is actually safe to eat, when the criteria have been met.

This concern has not prevented the continuing use of microbiological criteria in the European Union. In the new European Food Safety Regulation (Commission Regulation (EG) No 2073/2005), microbiological criteria have been set and relate to both food safety and process hygiene. In Table 2, an example is given here for ready-prepared fruit and vegetables.

Although there was always a rationale behind the setting of microbiological criteria, these must now involve a risk assessment. This is due to the view that risk management decisions should always be based on sound science⁸⁾. Particularly important is the fact that the WTO SPS Agreement requires a risk-analysis approach in food-safety assessment to protect consumers and stimulate international trade in a fair manner. It is recognised now that the prohibition and precautionary principles do not adequately reflect the actual risks involved, while process control criteria are mostly derived from worst-case scenarios. Also, microbiological criteria have little value in relation to pathogens that are required to be absent from a particular food.

The Risk Assessment Approach

Risk assessment comprises four building blocks (Fig. 1). The first is hazard identification,

Fig. 1. The 4 building blocks of risk assessment.

which refers to the microbes or their toxins that may be present in the product and have adverse effects on human health when the food is consumed. Then there is hazard characterisation. This deals with the characteristics of the hazardous agent as they affect the host and their relationship to the environment in which the agent occurs. For example, there is the ability of a particular organism to multiply in a food product, the infective capability of the organism, preferably expressed as a dose-response relationship, and the nature and severity of the disease caused. Exposure assessment is the third building block. At this step, the level of the hazardous agent in the food is assessed at the point of consumption. Information is also sought on the amount of potentially contaminated food that is consumed. Finally, the risk assessment is made, taking account of the level of hazardous agent in the food, the dose-response relationship and the possible severity of the illness. The outcome includes an estimate of the risk as a figure between 0 and 1. In practice, there are several different types of risk assessment. These include qualitative, deterministic and probabilistic approaches (Fig. 2).

Qualitative risk assessment is based mainly on experience and technical knowledge. The outcomes can be highly subjective, with considerable variation between different individuals making the assessment. Outcomes are expressed usually in terms of high, medium, low or negligible risk. On the other hand, the deterministic approach makes use of numerical models and generally produces an 'average risk'. This type of risk assessment has often been used to determine worst-case scenarios and in establishing codes of practice.

In contrast to the approaches just described, probabilistic risk assessment utilises probability distributions rather than conventional numerical data. In this way, variability in the

Fig. 2. The broad types of risk assessment.

data is taken into account. The approach allows quantitative scenario analysis and so-called mitigation studies to determine the effects of possible intervention strategies.

Let us consider how a risk assessment is carried out. This can be illustrated by reference to a qualitative/deterministic approach used in setting criteria for the occurrence of *Staphylococcus aureus* and its toxins in cheese. Here, the first requirement is to collect the necessary quantitative data, including the amount of cheese consumed per person, how often consumers are affected and the amount of toxin produced. It is also important to know the minimum toxic dose, which is in the region of $1\text{ }\mu\text{g}$ /portion consumed. This may be achieved when counts of *S. aureus* exceed 10^5 organisms per gram. The necessary information is presented in Table 3.

Based on these data, the new EU legislation (Commission Regulation (EG) No 2073/2005) has the following requirements:

- For controlling process hygiene criteria, 5 samples should be tested, of which 3 should contain $<10^4$ *S. aureus* per gram, while 2 samples may contain 10^4 to 10^5 per gram.
- In relation to product safety, if there are more than 10^5 *S. aureus* per gram, 5 samples of cheese should be tested for toxin and none should be positive.

An example of a probabilistic risk assessment concerns the presence of the pathogenic *Escherichia coli* O157 in pork production. In this, the first step is to make an outline of the entire process, up to the point of consumption (Fig. 3). Then, an assessment is made of the distribution of *E. coli* O157 levels in the live animal. This is followed by the construction of mathematical models to describe what happens at each stage of the process. An example of such a model is that produced for the effect of heat treatment on minced pork. Here, the meat is heated at 70°C for 20 seconds and 3 process variables can be recognised:

Table 3. Data needed for a qualitative/deterministic risk assessment for setting criteria for the occurrence of *Staphylococcus aureus* and its toxins in cheese

Cheese consumption data:
– 13–18 kg/capita/year
Foodborne disease data:
– Europe: 0,6% of outbreaks attributed to <i>S. aureus</i> and cheese
– US: 0,24% of all foodborne disease attributed to <i>S. aureus</i>
<i>Staph. aureus</i> food poisoning data:
– caused by enterotoxin of which several (sero/geno)-types exist
– most intoxications are caused by SEA
– minimal toxic dose $\approx 1 \mu\text{g}$ /portion consumed food
– this level may be reached after <i>S. aureus</i> exceeds $>10^5$ /ml

Fig. 3. The pork chain. Starting point in the risk assessment is to assess the probability distribution of *Escherichia coli* O157 in live animals (M1). By using models the effect of all processing stages is calculated. This results in a final contamination at point of consumption (M2).

- the initial level of *E. coli* O157 contamination (N_0)
- the heating time in seconds, and
- the D value, which is the time needed at 70°C to reduce the *E. coli* O157 count by 1 $\log_{10}$ unit.

As shown here, the resultant model is: $N_t = N_0 - 10^{(P70/D70)}$.

It has to be taken in mind that each variable has a probabilistic distribution. For example the initial level of *E. coli* O157 present varies from 0 up to $3.5 \log_{10}$ units/g product. This also applies to the heating temperature which may vary from 18–24 seconds. Also the D -values for *E. coli* O157 will be variable. The probability distributions need to be assessed for each variable (Fig. 4). These probability distributions are used in the model to calculate the number of cells of *E. coli* O157 that survive the heat treatment. The outcome is presented in Fig. 5. If the minced meat is consumed immediately after heating, the probability of becoming ill can be

determined by multiplying the final exposure distribution by the dose-response curve for *E. coli* O157. The outcome is the probability of illness within a certain confidence interval.

With probabilistic risk assessment, it is important to remember that not all foods are contaminated to the same extent by a particular pathogen and not everyone will prepare a food in the same way or eat the same amount. Also, it is necessary to take account of the differences in sensitivity to specific pathogens among different sectors of the population. This will introduce uncertainties in the final outcome of the risk assessment calculations. See among others the document on uncertainties in Dietary Exposure Assessment as produced by the European Food Safety Authority¹⁰.

Current Developments

About 10 years ago the Codex Alimentarius initiated a new approach to food safety (CAC, 2003). The overall objective was to set

Fig. 4. The variables, expressed in probability distribution curves, are the basis for assessing the effect of heat treatment of minced meat.

Fig. 5. The effect of the heat treatment, expressed in log cfu of *E. coli* O157/gram present in the minced meat.

food-safety criteria based on quantitative risk assessment. For this purpose, several new terms were introduced, such as 'appropriate level of protection' (ALOP), 'food safety objectives' (FSO), 'performance objectives' (PO), 'performance criteria' (PC) and 'process criteria' (PrC). The new approach is primarily based on setting an appropriate level of protection (ALOP). From this the food safety objective (FSO) the level of contamination that meets the ALOP is calculated. In turn from this FSO the performance objectives (POs), the performance criteria (PCs) and process criteria (PrCs) are calculated.

To explain this approach, we can take an example. Firstly, we assume that ready-prepared fruit and vegetables are causing an unacceptable level of foodborne illness, say 100 cases per 100,000 of the population per year. Therefore, a new appropriate level of protection (ALOP) has to be set. This is presented in Fig. 6.

To ensure that the ALOP is met, a new food safety objective (FSO) is needed and this is defined as the maximum permitted concentra-

tion of a hazardous agent in a food at time of consumption that contributes to the ALOP. In turn, the FSO must be translated into specific performance objectives, performance criteria and process criteria⁷⁾.

The best way to make the necessary calculations is to carry out a probabilistic risk assessment. This approach is particularly relevant when new intervention strategies are needed to meet the food safety objective. It allows a comparative evaluation of several such strategies and provides information on which a cost-benefit analysis can be made. The principles are presented in Fig. 7.

Conclusions and Comments

Probabilistic risk assessment yields the kind of data on which the risk manager can be sure to make the most appropriate decisions. This applies to food-safety criteria that are set by both legislators and food producers to ensure that the products in question are safe. However, the development of mathematical models is a very time-consuming activity. Also,

Fig. 6. An example: Reduction in disease from fresh produce.

Fig. 7. The way to derive a new food safety objectives (FSO) from a set target (or appropriate level of protection (ALOP)).

food production processes can be highly complex and variable in performance. Equally, too much attention can be given to the production side, whereas foodborne illness usually originates elsewhere.

The Codex Alimentarius approach²⁾ actually covers two separate domains. One deals with the legislative aspect, as in the setting of an appropriate level of protection (ALOP) or a food safety objective (FSO). The other is the domain of the commercial food producer, whose task is to use these criteria to develop suitable performance objectives, performance criteria and process criteria. Clearly, food producers must be responsible for their own control measures, while remaining innovative and competitive in the marketplace.

The one downside of the Codex approach is that it is difficult to communicate to those that need to use it, but this may improve with time and experience of the system.

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