

General Interest

Potential Application of Risk Assessment Techniques to Microbiological Issues Related to International Trade in Food and Food Products

INTERNATIONAL COMMISSION ON MICROBIOLOGICAL SPECIFICATIONS FOR FOODS (ICMSF) WORKING GROUP ON MICROBIAL RISK ASSESSMENT†

ABSTRACT

One of the components of the General Agreement on Tariffs and Trade Sanitary and Phytosanitary agreement that will have far-reaching effects on international trade in foods and food products is the requirement for countries to provide risk assessments as part of the process of resolving disputes that involve food safety issues. Risk assessment is a means of evaluating the likelihood and impact of hazards. It provides a framework for systematically considering available data, providing rationales for assumptions, and identifying areas where additional information is needed. While the application of quantitative risk assessment techniques to microbial food safety has been limited, recent studies have increasingly demonstrated its feasibility. Quantitative risk assessment is particularly well suited for use with the hazard analysis critical control point and appears to have potential as an approach for comparing the equivalence of international food safety programs and inspection systems.

One of the primary goals of all countries is to assure access to a food supply that is simultaneously nutritious, wholesome, abundant, affordable, and safe. As a means of meeting these sometimes conflicting goals, countries establish various food safety objectives and criteria. Similarly, food-manufacturing companies develop and employ a variety of ingredient and product specifications, processing requirements, and handling practices. Both can include microbiological guidelines or standards for raw ingredients or products prior to their use or entry into commerce. These requirements are not uniform around the world. Such differences can lead to trade disagreements among countries, particularly if it is believed that microbiological requirements that cannot be justified scientifically are being used as nontariff trade barriers for limiting access to a country's markets.

The means by which such disputes have been addressed have evolved over the course of the past 30 years. The historical approach has been to establish criteria through the deliberations of international bodies. The best recognized is

the Codex Alimentarius Commission that brings together representatives of member governments to debate and reach consensus on standards for foods in international commerce. The roots of the International Commission on Microbiological Specifications for Foods (ICMSF) are also in this era; it was originally established in 1962 under the auspices of the International Union of Microbiological Societies as a nongovernmental commission that could provide scientific advice on issues related to microbiological concerns influencing international trade in food. The ICMSF is composed of microbiologists from government, industry, and academia. It has fostered the scientific consideration of food safety concerns first through the establishment of statistically based sampling plans (27), and more recently the hazard analysis critical control point (HACCP) system (28).

While microbiological criteria have valid uses, it is often difficult to relate them directly to the array of microbial agents that can occasionally be associated with foods. Such limitations led to the development of quality/safety assurance systems, such as HACCP, that focus on verifiable process control. HACCP has proven to be a highly effective means for controlling foodborne biological, chemical, and physical hazards, but it is difficult to establish whether different HACCP plans can be compared in terms of equivalent consumer protection (6, 10, 41).

Judgements on microbiological food safety have typically been subjective, reflecting that it has seldom been possible to relate directly the microbiological status of a food to its likely impact on public health. However, food safety professionals are increasingly being called upon to find ways of making decisions based on more objective

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considerations of risk (24). This is most apparent internationally in the agreements reached as a result of the Uruguay round of the General Agreement on Tariffs and Trade (GATT). A key provision of the World Trade Organization Sanitary and Phytosanitary (SPS) Agreement is that when disputes among trading partners arise because of conflicting views about the safety of a food product, the countries are expected to provide risk assessments to help quantify whether the risks faced by consumers are significant or if the levels of assurance required from the exporting country are greater than those mandated by the importing country for its equivalent domestic industry. Such requirements for formal risk assessments are also finding their way into the laws of individual countries. For example, the United States' Department of Agriculture is now required to conduct a risk assessment and cost-benefit analysis for any new regulation classified as having a potentially major economic impact.

While political and economic forces are promoting the use of risk assessment techniques to evaluate microbiological food safety issues, this trend has to be counterbalanced by considering what techniques are available and practical. Despite the extensive development of quantitative and qualitative assessment methodologies for the evaluation of risks associated with chemicals, pharmaceuticals, environmental impact of technologies, and economic investment strategies, there have been only a limited number of attempts to apply quantitative techniques to the evaluation of microbiological concerns related to food safety and quality. However, this is changing rapidly as researchers and policy developers begin to explore potential applications with currently available techniques and develop new ones. The purpose of the current communication is to: (i) introduce practices used in quantitative microbial risk assessments, the scientific rationale underlying their selection, and how they differ from those used for chemical risk assessments; (ii) introduce some of the approaches and potential techniques that are being used; (iii) identify some of the limitations and potential pitfalls in developing quantitative microbial risk assessments; (iv) discuss potential applications and their implications following the successful development of microbial risk assessment techniques; and (v) identify knowledge gaps in methods and the types of data needed to conduct quantitative microbial risk assessments more effectively.

RISK ASSESSMENT AS PART OF RISK ANALYSIS

Risk assessment is one of three components of risk analysis, the others being risk management and risk communication (58). Put simply, risk assessment is the measurement of risk and the identification of factors that influence it. Risk management is the development and implementation of strategies to control that risk. Risk communication is the exchange of information pertinent to the risk among interested parties. Although each component represents a discrete activity, there are overlaps and interactions; each contributes to a dynamic, iterative risk analysis process (33).

There has been substantial debate about who should be responsible for the different components of risk analysis, particularly whether there should be a functional separation of risk assessment from risk management. In general, it has

been recommended that risk assessments be done by individuals or groups not involved in the subsequent management of the risk (58). The rationale behind this recommendation is that such separation helps to ensure that assessments are not biased by preconceived opinions related to management solutions, i.e., the assessors should focus on evaluating the system, not correcting it. However, often the risk managers are among the best qualified to evaluate scientific data pertinent to the risk assessment. In practice, effective risk assessments are best achieved through the use of multidisciplinary teams that can provide a broad consideration of the scientific issues, while minimizing bias in the selection and interpretation of scientific data (40).

Minimization of bias is also achieved by insuring that risk assessments are transparent (58). This term signifies that a risk assessment has been conducted in a manner where all assumptions, data, inferences, and conclusions have been fully communicated, and any areas of uncertainty have been clearly identified. The process can be greatly enhanced through the appropriate use of review panels, where a second group of experts critiques the conclusions reached by the initial assessment team and identifies areas that may have been overlooked. This approach helps protect the system from unrecognized bias or faulty assumptions resulting from an incomplete consideration of available data.

One reason for making risk assessments transparent is that it is extremely unlikely that all of the data and knowledge needed to achieve a complete assessment will ever be available. In fact, one benefit of conducting a formal risk assessment is that it provides the basis for considering immediate actions based on available information, while simultaneously identifying those areas where additional information would enhance risk management decisions (45). Risk assessments and the risk management decisions based on those assessments have a finite period before the assumptions and data used become obsolete. Any change in the production, distribution, or consumption of a food or the microorganisms associated with it has the potential for altering risk. The usefulness of quantitative microbiological risk assessment techniques is greatly enhanced when dynamic models can be developed as opposed to the snapshots in time that are provided by more traditional static risk assessments.

Quantitative risk assessment techniques have been used extensively in considering the chemical safety of foods. However, the direct transfer of these techniques is not possible because the basic assumptions underlying chemical evaluations differ markedly from microbiological evaluations, reflecting the unique attributes of each. Some of the characteristics that make most microbiological food safety concerns conceptually different from chemical concerns include:

(i) Microbial risks are primarily the result of single exposures. Each exposure to a pathogen or its toxin represents an independent, noncumulative event. Except for particular fungal toxins, there is little concern related to the chronic accumulation of a pathogen or microbial toxin, whereas with chemical assessments one of the primary

focuses is the cumulative effects of carcinogens and other long-term chemical toxicities. Even chronic sequelae associated with pathogens can be the result of a single exposure. If anything, multiple exposures over time can lead to the development of immunity and thus lessen overall risks.

(ii) While there do not appear to have been any direct comparisons, it is likely that the population's response to an infectious pathogen is more variable than to acutely toxic chemicals and rivals the complexity observed with carcinogenic compounds. The variability of response in large part reflects the variability in the immune status of humans. Individuals within the population can range from highly resistant to extremely susceptible depending on their genetics, age, physiological status, and a variety of other biological and socioeconomic factors that influence the complex systems that enable the body to protect itself against infection. In addition, because the disease process for infectious agents involves their multiplication in the host, there is often little correlation between the levels of the pathogen ingested and the severity of the disease response (17, 22). Secondary infections resulting from the person-to-person spread have to be considered when estimating the risks associated with highly infectious biological agents.

(iii) The levels of many toxic compounds in foods are relatively stable or decline over time as a result of degradation or dilution. There are few examples where the levels of toxic chemicals increase as a result of conditions of storage. In contrast, the levels of pathogenic microorganisms capable of growth in foods can change dramatically. Pathogenic bacteria can increase a billionfold in less than a day if allowed to grow as a result of abusive storage. Conversely, pathogen numbers can decrease a billionfold in minutes as a result of a simple cooking step. Because the formation of microbial toxins is linked to cell levels, the risk of foodborne microbial intoxications is also influenced by the conditions associated with food storage and handling. This potential for changes in pathogen or toxin levels and the accompanying need to consider the incidence of abuse greatly complicate the estimation of the key datum for an exposure assessment, i.e., the number of pathogen cells or amount of microbial toxin actually ingested by consumers.

(iv) Microorganisms are dynamic and adaptable. In addition to the potential for acquiring or losing virulence-associated characteristics, microorganisms have various physiological mechanisms that may allow them to adapt to control measures used to manage microbial risks. Two isolates of the same species can have highly disparate disease capabilities. For example, most *Escherichia coli* are nonpathogenic, but specific isolates such as *E. coli* O157:H7 are associated with life-threatening diseases. The virulence of isolates can also be affected by the food matrix in which they are present. Thus, microbial risk assessments must deal with the biovariability of the pathogen, the host, and the food. Risk assessments of food products and processes must be updated in response to the emergence of new pathogens or the reemergence of known pathogens with altered ability to grow or survive in foods or to cause disease.

COMPONENTS OF A MICROBIOLOGICAL RISK ASSESSMENT

A quantitative risk assessment produces a mathematical statement that links the probability of exposure to an agent and the probability that the exposure will affect the host. This is coupled with a consideration of the severity of the illness to yield an overall risk characterization. While different groups have subdivided the risk assessment process in various ways (13, 23, 33, 40, 58), typically four components have been described: hazard identification, exposure assessment, dose-response assessment, and risk characterization. The four steps of a risk assessment are similar for chemical and microbiological agents (43); however, the emphasis among the steps is likely to differ. For example, the focus during the hazard identification phase will be substantially different for a new chemical that is being considered for a food additive compared to a known pathogenic microorganism that may occasionally contaminate a food.

An important first step in conducting almost any risk assessment exercise is the development of a clear statement of both the objectives and scope of the risk assessment. In developing this statement there must be a continuing focus on the primary purpose for conducting a risk assessment, i.e., the systematic, objective acquisition and analysis of information that can assist risk managers in their decision-making. The statement of purpose should also provide a clear understanding of any restrictions that should be considered. For example, if the purpose of a risk assessment is to estimate the risk of gastroenteritis due to the presence of enteric bacteria in ground beef in country A, one would not include data on viral gastroenteritis in country B. However, pertinent scientific information generated in other countries or regions can be part of the scientific knowledge base used in the assessment. The statement of purpose should also specify the format of the answer or risk characterization (e.g., probability of infection, cases per 100,000) generated by the risk assessment.

Hazard identification. Hazard identification is a concept that is already familiar to the food industry. It is similarly equivalent to hazard analyses performed as part of an HACCP program. The focus of hazard identification varies depending on the end use of the risk assessment. If the focus is on a pathogen, then available epidemiological and related data will be used to identify if foodborne transmission plays an important role in the etiology of disease and which foods are implicated. Conversely, if a hazard identification is oriented toward the food, then the focus will be to use available epidemiological and microbiological data to determine which pathogens have been, or potentially could be, associated with the product. In both cases, the key to hazard identification is availability of both public health data and information on the occurrence and levels of pathogenic microorganisms in the foods of concern.

When considering the microbiological risks associated with a food or class of foods, typically several pathogens could be of concern. Conversely, when considering a particular pathogen, a variety of foods could potentially be

listed. At least some members of the team conducting a hazard identification must therefore have sufficient expertise to differentiate between trivial and nontrivial concerns and to identify commonality among concerns. For example, if the primary concern with a food is enteric pathogens derived from fecal contamination, then the most common or most resistant of this class of pathogens (e.g., *Salmonella*) may suffice as the focus of the risk assessment in terms of knowledge needed for subsequent risk management decisions. Similarly, the increasing use of *Listeria monocytogenes* as a target for assessing the risk associated with changes in hygienic practices is based on the knowledge that other pathogens are less difficult to control and thus represent a lower risk than the target organism. However, in both cases these are assumptions that must be elucidated clearly for the purposes of transparency. Likewise, such assumptions may change over time in light of new findings or new concerns.

Traditional foodborne pathogens are relatively well documented and the formal requirements for hazard identification are minimal. Three broad classes of foodborne pathogens (i.e., infectious, toxicoinfectious, and toxigenic) are differentiated based on the mechanisms underlying their pathogenicity. The following discussion focuses on the concepts associated with the first two classes. The relatively minor modifications required for the exposure and dose-response assessments that are needed when considering toxigenic pathogens are discussed later.

Exposure assessment. The second step in a risk assessment is estimating the probability that the pathogenic microorganisms selected by the hazard identification process are ingested by consumers. However, the exposure assessment cannot simply be the probability of the presence or absence of the pathogen but must estimate the numbers of the pathogen consumed by the population. The probabilities of infection, morbidity, and mortality increase substantially when the levels of the pathogen ingested are increased.

An accurate exposure assessment needs three different types of information: (i) the presence of the pathogen in the raw ingredients; (ii) the effect that food processing, distribution, handling, and preparation steps have on the pathogen; and (iii) consumption patterns. The occurrence of a specific pathogen is often sporadic and rarely distributed homogeneously in a food. Both the frequency and extent of contamination are needed, including factors that influence these levels such as seasonal and regional differences. Again, presence/absence data are not sufficient. Sufficient historical data on the levels in raw commodities or finished products are needed to provide an estimate of the likely distribution of a pathogen. It is often necessary to rely on data related to the raw ingredients of a food product because the levels of the pathogen in the finished product are too low to make surveys practical. In such instances the incidence in the finished product has to be inferred from the levels in the raw ingredients and the impact of the processing steps.

In the absence of large-scale studies such as the microbiological baseline studies conducted by the U.S. Department of Agriculture, groups of smaller studies can be

combined to estimate the microorganism's distribution. Included should be a clear indication of the lower limits of detection associated with the methods used to obtain the data and the statistical performance characteristics of the sampling scheme. One of the areas that has been identified as needing further study is interpreting the meaning of failing to detect the pathogen in survey samples. If a food container is truly free of a pathogen, as long as it is not recontaminated there is no potential for that product to be a source of the microorganism. However, unless every unit in a production lot is examined, it is not possible to guarantee that the food is pathogen free. Accordingly, when a pathogen is not detected in a survey sample, the level of the microorganism may not be zero but only below the lower limit of detection. Depending on the lower limit of detection, there is a finite possibility that the food could still be a source of the pathogen. Put simply, survey samples that indicate an absence of the microorganism represent two outcomes, true zeros and samples that contain levels of the microorganism that are below the detection capabilities of the methods and sampling plans employed. This latter group cannot be ignored because the risks associated with it could increase dramatically if the product was subsequently abused. Alternative statistical approaches are currently being explored to better handle these population distributions (37).

Each step in the manufacture and distribution of a food may have an impact on the levels of the microorganism of concern. Without an estimate of these effects it is difficult, if not impossible, to estimate the consumers' actual exposures. Historical performance data or laboratory studies can be used to establish the variability of all or part of a food process. Alternatively, predictive models of microorganism growth or survival responses to environmental conditions can be used to estimate microbial levels (2, 9, 11, 37, 55–57, 59–61). For example, mathematical models have long been used as a means of assessing the relative safety of thermal processes (56).

Once the extent of growth and/or survival of the pathogen during food production, distribution, and marketing has been established, the final phase of the exposure estimate focuses on consumer activities that affect microbial levels. Minimally, information on the average serving size is needed; however, incorporating data on the distribution of serving sizes and related attributes yields more accurate exposure assessments. This phase of the exposure estimate can be made more sophisticated by considering food preparation practices and consumption patterns that influence either the levels of the pathogen in the food or the amount of food consumed. For example, estimating the survival of pathogens in raw foods such as ground meats requires data on the distribution of cooking times and temperatures that are used by consumers. The physical distribution of the microorganism may have to be considered when estimating its thermal inactivation or growth as a microcolony within a solid matrix. Estimates of the extent of storage abuse can be incorporated into the exposure assessment to improve the estimate of consumer risks. Estimation of the total exposure over the course of the year would require additional

consumption pattern data on factors such as seasonality, age distribution, and regional differences.

The selection of models and data sets to be used in an exposure assessment is dependent on the purpose of the risk assessment. If the assessment is a nationwide evaluation of a food industry for broad policy considerations, then the data and models used will have to be general due to the great diversity in product formulations, production facilities, ingredient sources, distribution systems, and marketing options. However, if the purpose of the risk assessment is more focused, such as establishing critical limits in an HACCP program, then a modular unit operations approach has been suggested (9, 57).

When estimating the performance of a food production–distribution–marketing system, two principles of food microbiology and quantitative microbial risk assessment become important. The first deals with surviving fractions. Microbial inactivation kinetics are generally first order; the population declines in an exponential manner. For most processes this leads to calculated values of a fraction of a cell/gram. Such values cannot be assumed to be equivalent to zero. Instead, they are a statement of the probability of finding the microorganism in multiple units of the food. For example, if the calculated level of a microorganism is 0.01 CFU/g, this is equivalent to 1 CFU/100 g and roughly equivalent to finding one positive sample in 100 1-g samples. Inclusion of these surviving fractions is important for estimating the overall performance of a food operation. The second principle is that each microorganism, food, and food process has inherent variability that must be taken into account. Limiting the consideration of risk to a single mean or median value without some measure of variability can lead to significant errors in interpreting the safety of a process. For example, if two processes have the same average performance, the one with the greater variability will represent a greater risk because it has a greater likelihood of producing a product with either an elevated frequency or level of the pathogen of concern. A key result of using HACCP and its reliance on process control is a reduction in the variability of food processes (6, 28). Despite its average performance, a highly variable process would have an unacceptable level of risk.

Dealing with the combined variabilities inherent in multiple-step food production and marketing systems can be difficult but is critical to the development of realistic exposure assessments. The recent availability of computer simulation software that is powerful analytically but easy to use is changing rapidly the ability to deal with variability in a meaningful manner. These programs are based on Monte Carlo and latin hypercube techniques that employ a value-generating protocol that randomly selects a value for each step in the multistep process that has a distribution of potential values according to the specified variation for that step (34, 38, 49). A model is solved repeatedly until a profile of the likely performance of the multistep system begins to emerge. Such software allows some of the prior risk assessment assumptions and techniques to be evaluated more critically including the techniques by which worst-case scenarios have been determined and used (12). Computer simulations are also being used to enhance the dose–

response phase of microbial risk assessments. Potential implications of employing probability profiles instead of point estimates are discussed later.

Dose–response assessment. The response of a human population to exposure to a foodborne pathogen is variable, reflecting that the incidence of disease is dependent on the virulence characteristics of the pathogen, the numbers of the microorganism ingested, the general health and immune status of the hosts, and attributes of the food that alter microbial or host status. Each of these subjects will be discussed briefly.

Modern microbiology and molecular biology have established that in almost all instances the ability of a microorganism to cause disease is associated with the possession of one or more virulence characteristics. These include the synthesis of various toxins, the presence of attachment factors on the cell's surface, the ability to circumvent the host's immune response, and tolerance to adverse conditions and antimicrobials. Many of these characteristics are associated with extrachromosomal genes or are readily transferred among species. The relative virulence of strains of the same species or between closely related species can vary tremendously depending on the presence and expression of different virulence genes. For example, the relative pathogenicity of *Salmonella enteritidis* and *Salmonella pullorum* for humans differs by several orders of magnitude, even though these salmonellae have been traditionally considered closely related species.

The amount of a biological agent ingested strongly influences both the frequency and extent of the adverse effects produced by the pathogen. Increasing levels of a pathogen in a food will generally result in a greater percentage of the population becoming ill, a decrease in the time to the onset of symptoms, and may increase the severity of the disease in individuals. However, before evaluating specific relationships between dose and response, it is important to ensure that the data are describing the same response. In the case of enteric bacteria, three end points are most commonly measured: infection, morbidity, and mortality. The term infection is used and defined differently by various disciplines, so it is important to note that here it refers to the colonization of the intestinal tract by the microorganism. Both symptomatic patients and asymptomatic carriers are included in this definition. The terms morbidity and mortality signify, respectively, the portions of the exposed population that display symptoms and die as a result of the infection. Other end points can be used, but they should be clearly defined before attempting to establish the dose–response relationship. For example, in some instances it may be beneficial to establish the relationship between ingestion levels and the incidence of chronic sequelae such as reactive arthritis or hemolytic uremic syndrome.

When the logarithm of the number of bacteria ingested is plotted against the percentage of the population that becomes infected (i.e., colonized), a sigmoidal relationship is evident. This has been traditionally interpreted as indicating a threshold level below which ingestion of the organism does not produce infection or a disease response in the host.

This led to the concept of minimum infectious dose, the minimum number of bacteria needed to cause disease. There has been substantial effort to define the minimum infectious dose for various foodborne pathogens. However, an increasing number of scientists are challenging this concept and have been reevaluating the basic premise that such thresholds exist. An alternative hypothesis is that if one considers a large enough host population, the ingestion of a single pathogen cell has a finite possibility of causing an infection, and that this probability increases as the levels of the biological agent are increased (22, 46). For example, it has been estimated that a single cell of *Shigella* spp., a pathogen noted for its high infectivity, has a probability of 0.005 of causing an infection (17). Another way of expressing this concept is that if 1,000 people each consumed one *Shigella* spp. cell, five individuals in the group would become infected. The beta-Poisson and exponential distributions are two mathematical models that have been found to be useful for describing the dose-response relationship for different biological agents, particularly when low numbers of the agent are ingested (17, 22, 46). Both equations are nonthreshold sigmoidal functions. The nonthreshold character of the equations is more evident when the probability of a response is converted to log values (Fig. 1). The Weibull-gamma model has also been used as an alternative dose-response model (18).

Several areas associated with dose-response relations need further study to eliminate some of the uncertainty

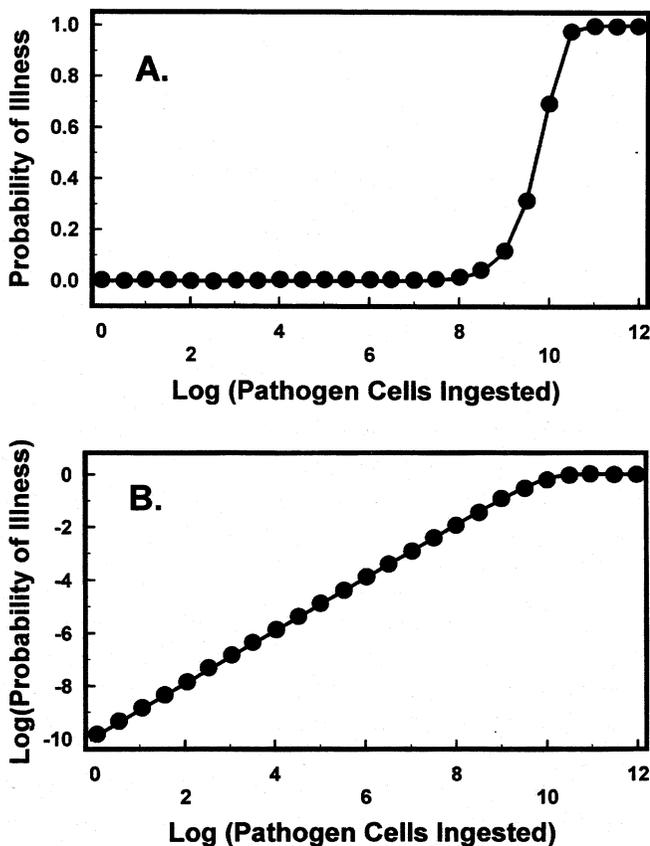


FIGURE 1. Exponential dose-response model when depicted as (A) $\log(\text{dose})$ vs. response and (B) $\log(\text{dose})$ vs. $\log(\text{response})$ graphs. Adapted from Buchanan et al. (7).

currently inherent in dose-response estimates. A key limitation to estimates of infectivity is that the values are largely based on a limited number of human volunteer studies. The volunteers in these studies were, by necessity, restricted to healthy individuals. Potentially, this underestimates the risks faced by more susceptible portions of the population. A second closely related area is the validity of extrapolating dose-response relationships for the general population to immunocompromised segments of that population. It has been estimated that the immunocompromised, including the very young and the elderly, may represent as much as 20% of the total population (13, 50). It is unclear whether suppression of the immune system makes individuals more susceptible to initial infection or if the infection rates are similar, but there is a greater likelihood that infected individuals become symptomatic. In statistical terms, it is uncertain whether the different classes of immunosuppressed individuals represent one of the tails of the response distribution for the general population or a statistically separate population. Not only is this important for more accurate dose-response estimates, there are also implications for risk management options. Several alternative approaches, such as animal models and worst-case dose-response assessments, have been suggested for estimating dose-response relationships for pathogens that are not amenable to human volunteer feeding studies (7).

The food matrix in which a pathogen resides can substantially modify the dose-response relationship for pathogens transmitted in food and water. This largely reflects survival of the microorganism as it passes through the hostile environments associated with the upper portion of the human gastrointestinal tract, particularly the stomach. The acid conditions in the stomach are the body's first defense against oral infection. Anything that increases stomach pH, decreases the microorganism's exposure to the acids in the stomach, decreases transit time, or increases a pathogen's acid tolerance decreases the dose needed to produce infections. Examples of pathogen- and host-related factors associated with increased survival in the stomach include decreased acid production resulting from age, use of antacids, preexposure of bacteria to moderately acidic conditions, entrapment of microorganisms in lipid droplets, and initial rapid transit of liquids when consumed on an empty stomach.

Toxicogenic microorganisms. While the overall risk assessment approach outlined above for infectious and toxicoinfectious microorganisms is the same for toxicogenic pathogens, some modification is needed to exposure and dose-response assessments because of their mechanisms of action. These species cause disease due to the action of preformed toxins causing acute toxicities (e.g., *Staphylococcus aureus* enterotoxin, *Clostridium botulinum* neurotoxin), chronic toxicity, or carcinogenicity (e.g., aflatoxins) associated with specific compounds produced by the biological agent. The extent of disease relates to the levels of toxin ingested by consumers. However, microbial levels are important because toxin production is linked to microbial proliferation. Thus, the exposure assessment must consider

both microbiological and chemical attributes, while the dose-response assessment reverts to consideration of the toxin. Unlike the infectious and toxicoinfectious pathogens, some of these organisms display population thresholds that must be reached before there is a host response (e.g., *S. aureus*). For extremely potent substances, the mere presence of the toxin may be deemed unacceptable (e.g., botulinum neurotoxin).

Risk characterization. Risk characterization is the integration of the exposure and dose-response assessments to provide an overall probability of consumers being subjected to infection, morbidity, mortality, or whatever biological response is being considered. Risk characterization should include a description of statistical and biological uncertainties. Assessments performed using dynamic models based on Monte Carlo techniques would include appropriate analyses such as probability profiles, sensitivity analysis, etc.

EXAMPLES OF QUANTITATIVE MICROBIAL RISK ASSESSMENTS

Until recently, there have been few published risk assessments that have attempted to evaluate quantitatively the microbiological safety of food or water. Most of the early studies focused on drinking water, reflecting an initiative by the U.S. Environmental Protection Agency to establish drinking water standards on a more scientific basis (36). The impetus for this initiative reflects the need to assess the relative risks associated with bacterial, viral, and protozoan contamination against those associated with use of the disinfectant chemicals (e.g., chlorine) used to eliminate them. The general approach was to establish a target tolerable risk (e.g., less than one *Giardia* infection per 10,000 people per year) and then to conduct a risk assessment to evaluate the ability of current water treatment approaches to meet that goal. A series of risk assessments done for enteric viruses, bacteria, and protozoa (21, 46, 47) were used as the basis for a cost-benefit analysis of pathogen control programs for drinking water (14). While an important step in establishing the efficacy of quantitative risk assessment, these initial quantitative assessments largely focused on establishing dose-response relations and were relatively simplistic in relation to exposure assessments (20). They did not consider factors affecting exposure such as pathogen distributions in the raw water and the changes in pathogen levels likely to occur as a result of water treatment and distribution.

An extension of this approach was used to conduct a quantitative assessment of the risk of acquiring a viral infection from the consumption of contaminated raw shellfish (48). A nonthreshold model for infection was employed for the dose-response assessment that included a consideration of the probability of infection, morbidity, and mortality. Subsequently, Todd and Harwig (52) conducted a series of semiquantitative risk assessments to characterize the risk associated with the consumption of four food classes.

One of the first attempts to assess the microbiological risks associated with a food process was a quantitative hazard assessment for *L. monocytogenes* in milk processing

that was conducted to evaluate the efficacy of current milk production and pasteurization practices (44). Six key production or processing factors were identified: concentration of *L. monocytogenes* in subclinically infected cows, fraction of herd infected, increase in contamination caused by poor sanitation, fraction of farms with infected cows, increase in levels during storage, and inactivation by pasteurization. Using this hazard assessment, the investigators concluded that there was less than a 2% probability that one *L. monocytogenes* would occur in 5.9×10^{10} gallons of pasteurized milk. Cassin et al. (12) commented that this value was likely an overestimation of the risk due to the methods used to calculate overall risk. They recommended the use of Monte Carlo simulation techniques to achieve a more accurate estimate.

Buchanan and Whiting (9) proposed that dynamic risk assessment models could be developed to link exposure and dose-response models. Using a hypothetical example, they demonstrated that initial raw product pathogen distribution data could be coupled to predictive microbiology models to provide a dynamic exposure model. The results from the exposure model then served as the input for dose-response models. They further demonstrated that this approach could be used to characterize complex, multiple-step processes using Monte Carlo techniques. While different terms have been coined by various investigators (e.g., process risk model (11), dynamic fault tree model (37)), this unit operations approach has been the basis for many of the quantitative microbial risk assessments reported recently. They have in common the use of a series of probabilistic and stochastic predictive microbiology models to describe factors affecting exposure and then using the result of the exposure assessment as the input for dose-response models as a means of linking exposure to human health impact.

The subjects of recent, increasingly sophisticated attempts to quantify the risks associated with various foods and foodborne pathogens reflect areas where there have been substantial food safety concerns, and in some cases disagreements, among international trading partners. Three areas that have received a great deal of attention are *S. enteritidis* in eggs and egg products, *L. monocytogenes* in ready-to-eat foods, and enterohemorrhagic *E. coli* in ground beef.

The simultaneous emergence of egg-associated *S. enteritidis* outbreaks in Europe and North America has stimulated several attempts to identify and quantify the risk factors that contribute to the incidence of human health problems. The sporadic nature of contamination in combination with the complexity of how eggs are produced, processed, distributed, prepared, and consumed has led to a need for methods to compare control strategies to determine which are likely to be effective both in terms of public health assurance and economics. Using production, survey, and epidemiological data, Todd (51) used a traditional risk assessment approach to evaluate the increased risk associated with the use of cracked eggs in Canada. This included an assessment of potential risk management options. As a means of demonstrating the use of predictive microbiology to achieve dynamic microbial risk assessment models, Whiting and Buchanan (57) used the unit operations approach outlined above to

quantify the risk of acquiring an *S. enteritidis* infection from homemade mayonnaise made from pasteurized liquid whole eggs. This 11-step model incorporated factors from farm (e.g., % of infected flocks) to consumer (e.g., serving size, duration, and temperature of home storage). The model allowed the effects of changes in a variety of environmental, formulation, and raw material variables on the infection probability profile to be calculated readily. More recently, a multidisciplinary team from the U.S. Department of Agriculture (2) has completed the initial selection of variables and models for an assessment of the risks of acquiring salmonellosis from a variety of egg and egg products. This assessment expands previous efforts by considering a range of products and practices and by the inclusion of cost-benefit estimates for different control strategies.

The establishment of quantitative microbiological criteria for the presence of *L. monocytogenes* in ready-to-eat foods in international trade has been the subject of substantial debate during the past several years. The lack of data indicating clearly defined threshold values for *L. monocytogenes* infections among susceptible populations has hindered the acceptance of nonzero criteria. Because it is unlikely that such dose-response data can ever be acquired (7), alternative approaches to determining dose-response relations have been investigated (7, 18) to estimate the risks associated with different levels of contamination. Several qualitative and semiquantitative assessments of survey and epidemiological data have suggested that the risks associated with the low levels of *L. monocytogenes* often found in ready-to-eat foods are so minimal that they are inconsequential in relation to public health strategies (7, 18, 25). Based on a risk evaluation of these data and assessments, ICMSF (30) proposed to the Food Hygiene Committee of the Codex Alimentarius Commission that a microbiological criterion of 100 CFU/g be established for *L. monocytogenes*. Miller et al. (39) demonstrated how a combination of microbial risk assessment models, predictive microbiology models, and simulation modeling techniques could be used in HACCP programs to establish critical limits and other process criteria that would assure that the microbiological criterion proposed by ICMSF would be consistently met.

The association of highly infectious enterohemorrhagic *E. coli* with ground beef has also stimulated the use of risk assessment and predictive microbiology techniques to estimate the importance of different risk factors and to evaluate the impact of potential control strategies. Marks et al. (37) developed a dynamic quantitative risk model for factors contributing the overall risks associated with the production and consumption of ground beef. Cassin et al. (11) developed a dynamic model for ground beef production and consumption; however, they expanded their model to include factors that contribute to the contamination of beef during slaughter operations. Zwietering and Hasting (60, 61) modeled the steps in the ground beef manufacturing operation using process engineering simulation modeling in combination with predictive microbiology models to quantify the contributions that individual steps have on the overall risks associated with the process. They concluded that this approach would be applicable to both the develop-

ment of quantitative microbial risk assessments and the establishment of critical control points and their critical limits for HACCP programs.

In addition to the three pathogens above, a limited number of quantitative microbial risk assessments have been attempted with other microorganisms. Zwietering et al. (59) used predictive microbiology and risk assessment modeling techniques to evaluate the factors affecting the incidence of *Bacillus cereus* in pasteurized milk at the time of consumption. Berends et al. (3-5) used a risk assessment approach to evaluate the factors influencing the incidence of *Salmonella* in pork carcasses. In both cases, the investigators used these techniques as tools to better quantify the hazard analysis phase of HACCP programs and evaluate the potential impact of changes in food safety inspection systems. Similarly, van der Logt et al. (53) conducted a microbial risk assessment to determine the public health protection achieved by examining New Zealand beef for the presence of *Taenia saginata*.

These studies demonstrated that by combining risk assessment and predictive microbiology models, it is possible to generate risk assessment models that can adequately address the complexity associated with the production, processing, distribution, and consumption of foods. In a few short years, microbial risk assessment has gone from being a concept to a tool employed actively to evaluate food safety risk management options.

FUTURE RESEARCH NEEDS

Despite the assumptions that had to be made in the examples above, the models developed are useful for running simulations and sensitivity analyses to identify the effects of changing different components on the resulting prediction of risk. However, for quantitative risk assessment to gain credibility, wherever possible the underlying assumptions should be validated and justified. The areas of research needed to generate data for validation are discussed below.

In the area of exposure assessment there are extensive data on the microbial ecology of particular raw materials and foods (26, 29, 31), but these data are often incomplete or inadequately quantitative. There is a need for information that better characterizes and follows those strains associated with foods and foodborne disease. The numbers and probability of pathogens contaminating food at different stages of the food chain are poorly documented and certainly not quantitative. This is especially true for very low pathogen levels where data are very sparse. The development of more sensitive, quantitative analytical and statistical methods are needed to detect reliably and characterize low numbers in foods. In predictive microbiology there are some excellent models that describe the effects of different parameters on growth of pathogens and that have been validated in foods (56). However, there is a need to develop further models for survival and inactivation of pathogens under different conditions. There is also a need to understand the risk implications of nonfirst-order inactivation kinetics that have been observed in numerous studies (1, 8, 15, 35, 55). An important consideration for both growth and inactivation modeling is better estimates of inherent within- and between-strain biovariability. This will be essential for the application of

predictive models to very low pathogen levels. Single cell techniques such as flow cytometry and image analysis (16) will help understand variation of responses in microbial populations. Another area where better information is needed is in relation to consumption patterns and consumer practices.

For dose–response assessments, estimating the numbers of pathogens required to cause infection has been traditionally an extremely difficult area in which to acquire good data. Data from outbreak investigations are generally incomplete, human feeding studies are limited, and the relevance of animal studies to the human dose–response is often questioned. It is very likely that assumptions in this area will still have to be made. The use of conservative dose–response values based on available epidemiologic and food survey data has recently been proposed as an alternate approach for those pathogens that are not amenable to human volunteer feeding trials (7, 18, 54). There is a need for research on the virulence factors and disease mechanisms of pathogens and how they are influenced by factors associated with food and food processing. Likewise, a better understanding of infection processes and the factors that influence the outcome of exposure is needed to estimate more effectively the range of host susceptibilities. Better understanding of the susceptibility of high risk groups continues to be a priority research need in risk assessment.

For toxigenic pathogens there is a need for additional quantitative data on the relation between the extent and conditions of microbial growth and the amount of toxin synthesis. Likewise, dose–response studies are needed to correlate better the relationship between levels of toxin ingested and the symptoms produced.

QUANTITATIVE MICROBIAL RISK ASSESSMENT AND HACCP

While much of the focus on quantitative microbial risk assessment has been directed toward its application on broad issues, a number of investigators have discussed its relationship and potential application to HACCP. There can be substantial confusion concerning the difference between risk assessment and HACCP because the first phase of HACCP is the identification of hazards (19). However, HACCP is primarily a risk management system (10, 19); thus, the role of quantitative microbial risk assessment is to provide the information HACCP system developers need to make more informed decisions. Microbial risk assessment techniques and data are already being studied for their potential uses in HACCP systems development (2, 9, 42, 57) and efforts to modernize inspection systems for foods in international trade (5, 23, 53). In addition to enhancing the hazard analysis phase of HACCP, risk assessment modeling techniques and the accompanying ability to relate levels of microbial control to public health effects can be used to assist in the identification of critical control points (CCPs) (5, 42, 57), the establishment of critical limits (9, 10, 41, 57, 60, 61), and determination of the disposition of product produced during periods of CCP deviation (10, 57). Whiting and Buchanan (57) proposed that the establishment of scientifically derived critical limits for HACCP will most

likely involve an iterative process of measuring risks and evaluating risk management options (e.g., modifying processing parameters, altering storage conditions) until a company has confidence that it can consistently produce a food that meets food safety requirements.

IMPLICATIONS AND LIMITATIONS

The ability to conduct quantitative microbial risk assessment techniques will change the way governments and the food industry conduct business internationally. Its greatest initial impact is likely to be in the areas of equivalence and harmonization. Through the efforts of the Codex Alimentarius Commission and other international bodies, there have been attempts to harmonize international guidelines and standards. However, confusion exists about the process of harmonization (23). Harmonization is based on the concept of equivalence, a term used to convey the fact that there are typically several ways of assuring that a food provides the same level of consumer protection. As international trade of foods is considered increasingly under the auspices of the SPS Agreement, assessment of the equivalence of food safety protection systems will become a key issue. Currently, this is done by a qualitative evaluation process and bilateral discussions, and these approaches will continue to be the major means of reaching consensus on food safety standards. However, as the ability to measure the risks associated with foods increases, there will be an increased reliance on quantitative analyses, particularly when dealing with issues that are contentious. In any discussion of scientifically based consideration of food safety risks, sound quantitative analyses provide much stronger evidence than qualitative evaluations. The potential importance of risk assessment in international trade is reflected in the accelerated development of guidelines for risk assessment and risk management that is currently underway by the Codex Alimentarius Commission.

Perhaps the most important implication of the ability to describe quantitatively the risks associated with foods will be to emphasize dramatically that no food is risk free and that each step in the farm-to-table continuum has a role in assuring its safety. This, in turn, implies a need to establish tolerable risk levels. The establishment of such criteria must take into account public opinion, technological and economic feasibility, and international consensus but must be based on sound science. This will also require consideration of questions such as how the risks should be managed, who should manage them, and who should pay for it. Such a process will require that the risk managers be able to work iteratively with risk assessors and stakeholders to evaluate the impact of potential risk management options (33). Further, the ability to compare quantitatively the food safety status of different foods is likely to lead to pressure to adopt risk management approaches that are open, transparent, and more consistent between food classes.

One of the challenges facing the development of food safety risk management systems based on risk assessments is the need to consider risk distributions. While sampling plans have been developed to consider the variability associated with the incidence of pathogenic bacteria in

foods, most food safety or microbiological criteria established by both national and international bodies have been single values (e.g., $<10 E. coli/g$, $<1 L. monocytogenes/25 g$). However, as techniques in quantitative microbial risk assessments become available, particularly simulation modeling methods, risk managers will have to analyze and interpret risk distributions that take into account both the inherent variability of biological systems and the uncertainty of the data available. For example, instead of stating that there is a zero tolerance for a specific pathogen, a risk-based criterion might more accurately indicate that there was $>99\%$ confidence that the level of the pathogen was <1 CFU/kg.

The ICMSF is currently exploring a six-step process for the management of food safety risks that is based on microbial risk assessment but simultaneously takes into account the diversity that is characteristic of the food industry worldwide (32). The initial step is a risk assessment that relates the levels of a microbiological concern to its effect on public health. These data serve as the scientific basis for discussions between governments and stakeholders to establish food safety objectives. Such objectives specify tolerable levels of risk, are quantifiable, and are measurable, either directly or indirectly. Governments then use the food safety objective to develop both processing criteria and performance criteria. The former are based on the overall capabilities of the industry and provide, through good manufacturing practices and processing guidelines, conservative assurances that a food safety objective is always met. Performance criteria achieve the same end but also provide the basis for alternatives to processing criteria established by regulatory authorities when a company can demonstrate that a different approach will satisfy the food safety objective.

An implication arising from the demonstration that zero risk is unobtainable is the need to communicate this reality to the public in easily understood, rational terms. Further, the use of risk assessment tools to evaluate the impact of feasible risk management options is likely to demonstrate for some issues that modification of consumer practices is the key risk management strategy. Both areas will require increased need for more effective risk communication and reinforce the need for innovative approaches to consumer education. Similarly, consumer education is needed related to international trade issues such as equivalence and alternative inspection systems.

As discussed above, risk assessment will be increasingly integrated into HACCP programs. In particular, it has the potential for becoming an important means of evaluating HACCP systems in relation to establishing the equivalency of HACCP programs both within an industry or between countries. Most regulatory food inspection programs include the control of aesthetic defects that are unrelated to food safety. This will become more apparent as regulators move toward using risk assessment and human health outcomes as a measure of food hygiene programs. This should help facilitate trade by providing a framework around which international consensus can be reached on the types of changes needed in inspection programs. Some of the initial microbial risk assessments that have been reported were

undertaken to assess the efficacy of inspection practices (2, 5, 53).

An important product of a formal risk assessment is the identification of critical data that are lacking. The increased use of these techniques will impact the direction and focus of public health programs, food safety research, and the types of information collected through surveillance programs. The transition from qualitative to quantitative microbiological risk assessments will require input from a variety of different sources. This is likely to stimulate increased sharing of scientific data among government bodies, industry, academia, and international organizations, including the development of new systems for data acquisition and access. A few systems, such as the Internet-based system for sharing international epidemiological data related to *Salmonella* outbreaks, are already underway. For many pathogens and foods, an abundance of information has already been published. However, these data have been generated using a plethora of sampling and testing methods that vary in statistical validity, specificity, and sensitivity. Although these data should not be ignored, care must be applied in integrating data sets. This need for data consistency is likely to provide additional support for efforts to establish international standards for microbiological methods.

The strengths and limitations of the risk assessment process must be understood by those who use this tool for decision making. Risk managers must understand what risk assessment can and cannot do. This includes understanding current limitations and being aware of new applications that are being developed. However, it must always be appreciated that risk assessment is a tool for facilitating sound decision making and is not a substitute for sound judgement. Poor decisions arising from the inappropriate use or interpretation of these techniques would greatly hinder the acceptance of the risk assessment process.

A temporary limitation to the application of quantitative microbial risk assessment is that most scientists involved with food safety research have not been trained in these techniques. However, this can be circumvented through the establishment of assessment teams and by drawing on the expertise of statisticians and risk assessors from other disciplines. The continuing development of user-friendly software that will facilitate the risk assessment process can also be anticipated. However, the typical limiting factor in performing an effective quantitative microbial risk assessment related to a food safety concern is not in the application of mathematical techniques; it is the lack of available expertise and data in food microbiology.

Risk assessment is based on the identification and characterization of hazards. As such, it cannot predict newly emerging microbial threats to human health associated with foods. Assessing the risk associated with a new agent will continue to require the acquisition of epidemiological, clinical, and microbiological data needed to characterize the pathogen. However, the inclusion of risk assessment techniques early in the investigation will foster a systematic approach to setting priorities for the information required at different stages.

Advances in the field of predictive modeling, comput-

ing, analytical microbiology, and epidemiology have made it possible to begin performing quantitative microbial risk assessments. Without doubt, these techniques have the potential to change the way in which the safety of foods is assessed and managed by policy makers and industry. This capability may offer, for the first time, a structured scientific process that can achieve goals such as being able to relate critical limits for HACCP systems to public health outcomes. Because of the SPS Agreement and the role of the Codex Alimentarius Commission in setting international standards and guidelines, food safety issues associated with international trade are likely to be the proving grounds for this new tool. However, the true potential of quantitative microbial risk assessment will only be realized through the integration of the expertise of food microbiologists, veterinarians, epidemiologists, medical scientists, and biometricians from government, public health authorities, industry, and academia.

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