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Microbial risk assessment: dose-response relations and risk characterization

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Abstract

Characterizations of the risks associated with foodborne pathogens are dependent on the availability of information on the population's exposure to the biological agents. However, by itself, exposure data are insufficient to assess the public health impact of pathogenic microorganisms. This requires the availability of effective dose-response models. Successful development of models that describe dose-response relations for enteric pathogens is dependent on a sound understanding of the mechanisms of pathogenicity associated with individual pathogens. This includes knowledge of how the various pathogen, host, and food matrix factors influence pathogenicity. Currently, a group of sigmoidal mathematical equations are used to empirically describe dose-response relations. While these have proven to be highly useful, advances in microbial food safety risk assessment will likely require the development of mechanistic models that more effectively consider the range of factors that influence the frequency and severity of foodborne infections in a population.

Keywords: Dose-response; Foodborne pathogens; Mathematical models; Pathogenicity

1. Introduction

In the preceding manuscript (Lammerding and Fazil, 2000), two of the four steps (i.e. hazard identification and exposure assessment) associated with the general framework for conducting food safety risk assessments were explored in relation to the evaluation of food safety risks of a microbiological nature. The purpose of the current manuscript is to continue this examination of the emerging field of microbial food safety risk assessment by considering the remaining two steps, dose-response assessment and risk characterization. This will include an in depth consideration of dose-response modeling and a general consideration of potential pitfalls associated with risk characterization.

This manuscript will focus on principles and techniques related to quantitative microbial food safety risk assessments. Qualitative risk assessments and hazard evaluations are currently more commonly used than formal quantitative assessments. There are a number of specialized techniques for such qualitative evaluations. However, the rapid development of quantitative techniques is accelerating the adoption of that more rigorous consideration of food safety risks. Further, qualitative risk assessments can

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be viewed as quantitative assessments where the degree of uncertainty is too great to allow the assignment of other than broad categories of variable weights (e.g. high versus low risk). It is interesting to note that in almost all qualitative risk assessments reviewed by the authors, at some point the descriptors were converted to numeric values for the purposes of further analysis, thus converting what had been designed to be qualitative assessments into quantitative assessments.

The scope of the manuscript will be largely limited to a consideration of foodborne diseases caused by bacteria. While there are a variety of non-bacterial foodborne pathogens (e.g. viruses, protozoa, toxigenic fungi, parasites), available information related to microbial food safety risk assessments, as well as the factors influencing pathogenicity and the disease state, are generally greater for pathogenic bacteria.

2. Dose-response assessment (hazard characterization)

The response of a human population to exposures to a foodborne pathogen is highly variable, reflecting the fact that the incidence of disease is dependent on a variety of factors such as the virulence characteristics of the pathogen, the numbers of cells ingested, the general health and immune status of the hosts, and the attributes of the food that alter microbial or host status. Thus, the likelihood that any individual will become ill due to an exposure to a foodborne pathogen is dependent on the integration of host, pathogen, and food matrix effects. These interactions are often referred to as the infectious disease triangle. Each of these classes of factors will be discussed briefly.

2.1. Microbiological factors

Any consideration of microbiological dose-response relations must take into account the various modes of pathogenicity associated with different pathogenic foodborne bacteria. Without an understanding of how a pathogen causes disease, it is difficult to evaluate and interpret the host and food matrix effects that influence pathogenicity. Three broad classes of foodborne pathogens are differentiated, infectious, toxico–infectious, and toxigenic, based on their modes of pathogenicity. Infectious agents typically have a three-step process by which they elicit a disease response; ingestion of viable cells, the attachment of these cells to specific locations along the gastrointestinal tract (or some other mechanism for avoiding being swept away due to peristalsis), and the invasion of either the epithelium (gastroenteritis) or the body proper (septicemia). Toxico–infectious agents follow a similar three-step process, except that instead of invading the epithelium or body, they remain in the gastrointestinal tract where they either produce (e.g. *Escherichia coli* O157:H7) or release (e.g. *Clostridium perfringens*) toxins that affect sites on the epithelium and/or within the body. This difference can substantially influence dose-response relations. By remaining in the intestinal tract, these microorganisms avoid many of the host's defenses, but have to compete for a niche within the intestinal tract. Toxigenic bacteria are differentiated on the basis that they cause disease by producing toxins in foods prior to its ingestion. With some toxigenic bacteria (e.g. *Staphylococcus aureus*), it is not uncommon for the food to cause disease due the presence of pre-formed heat stable toxins despite the fact the microorganism was destroyed by food preparation steps subsequent to its growth in the food. For toxigenic microorganisms dose-response relations are essentially that for a chemical toxin. In some instances toxigenic bacteria can behave as toxico–infectious pathogens. For example, *Clostridium botulinum* is classically considered a toxigenic foodborne pathogen that causes disease due to the presence of pre-formed neurotoxin in a food. However, infant botulism involves the colonization of the intestinal tract and subsequent production of the toxin in the host. The differences in these modes of pathogenicity for the same pathogen would be expected to make the dose-response relations for the two disease syndromes substantially different.

One of the key questions that must be asked when evaluating, or even more importantly when comparing dose-response studies is what biological response is being measured. In the case of pathogenic enteric bacteria, three biological endpoints 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 for

enteric pathogens this term is generally used to refer to the colonization of the intestinal tract. Both symptomatic patients and asymptomatic carriers are included in this definition. The terms morbidity and mortality signify, respectively, the portions of the individuals infected that display symptoms or die as a result of the infection. Other endpoints can be used, but they should be clearly defined before attempting to establish a 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.

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 can include a variety of physiological capabilities such as the synthesis of a toxin, the presence of attachment factors on the cell's surface, the ability to circumvent the host's immune response, or the resistance to adverse conditions and antimicrobials. Often these characteristics are associated with extrachromosomal genes or are readily transferred among species. The relative virulence of strains of the same species or that of 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 *S. pullorum* for humans differ by several orders of magnitude, even though these salmonellae have been traditionally considered closely related serovars (Coleman and Marks, 1998). Similarly, a number of different disease conditions are associated with specific *E. coli* isolates (e.g. enterohemorrhagic *E. coli*, enterotoxigenic *E. coli*), whereas the majority of biotype 1 *E. coli* isolates are considered non-pathogenic. Even when two isolates possess all of the same virulence characteristics, there can be substantial differences in pathogenicity due to differences in the expression of the characteristic (e.g. toxin synthesis).

In addition to the specific virulence characteristics associated with individual strains of a pathogenic bacterium, the number of the cells 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. As will be discussed in more detail later, this is not usually a linear relationship. Increasing the number of pathogen cells ingested also tends to reduce the mean 'incubation period' for enteric diseases, i.e. the time between the ingestion of the pathogen and the onset of symptoms. This is likely due to the increased levels of the pathogen decreasing the time needed to overcome the host's defenses and cause physiological damage. The relationship between initial dose and the severity of the disease is less clear-cut. For example, Medema et al. (1996) reported that while the rate of infection for *Campylobacter jejuni* was dose related, the incidence and severity of morbidity was not. Alternatively, it has been suggested that there is an increased rate of severe *Salmonella* infections at higher doses (Coleman and Marks, 1998). Plausible mechanistic arguments can be made why both conclusions could be correct. As will be discussed further below, the response of individuals to a pathogen is highly dependent on their physiological status. It is possible that high doses result in multiple sites of infection within an individual's intestinal tract, thereby amplifying the impact of the pathogen. Alternatively, it is feasible that severe infections are only observed with individuals with predisposing conditions (e.g. depressed immune system). At low doses the number of infections observed may be insufficient to have an adequate number of observations to accurately determine the relationship between dose and severity. This would be particularly true for human feeding trials where the number of observations are very low.

2.2. Host factors

The second leg of the disease triangle are factors associated with the host that can influence individuals' susceptibilities to foodborne pathogens. Human populations are highly diverse in relation to their response to infectious agents, reflecting the population's diversity in terms of genetic backgrounds, general health and nutrition status, age, immune status, stress levels, and prior exposure to infectious agents. For certain foodborne disease, it appears that prior exposure to the agent renders the individual resistant to subsequent exposures to the pathogen (e.g. *Cyclospora cayetanensis*). However, for many infectious and toxico–infectious foodborne pathogens, immunity is of limited importance due to

either the presence of the pathogen being restricted to the intestinal tract (e.g. enterohemorrhagic *E. coli*) or that there is a great diversity of serotypes (e.g. *Salmonella*).

There are segments of the populations that are at increased risk. This appears to be associated, in large part, with these groups having depressed immune capability. Typically, the very young and the elderly are considered at increased risk of foodborne infectious agents, reflecting their immature and reduced immune responses, respectively. Likewise, medical interventions (e.g. immune suppressive drugs) or disease states (e.g. HIV) that adversely affect the immune status or overall health status can influence incidence or severity of foodborne diseases. It has been estimated that the immunoimpaired, including the very young and the elderly, may represent as much as 20% of the total population (CAST, 1994; Gerba et al., 1996; Smith, 1998), though it is unclear whether this entire segment of the population is at increased risk for any specific pathogen. It is also unclear if depression of the immune system makes individuals more susceptible to an initial infection, or the infection rates for immunoimpaired and fully immunocompetent are similar but there is a greater likelihood that infected individuals become symptomatic. In terms of dose-response modeling, it is unclear whether it would be better to treat the different groups of immunoimpaired individuals as the 'tail' of response distribution for the general population or as distinctly different populations.

2.3. Food matrix factors

The final leg of the disease triangle is the influence that the food in which the pathogen is transmitted has on dose-response relations. Previously, food was generally viewed as a neutral vehicle for the pathogen and as such had little impact on dose-response relations. However, during the past few years there has been an increasing awareness of the magnitude of the impact that food matrix effects can have on the likelihood of disease. Much of the focus has been on the impact that microbial adaptation has on the acid resistance of enteric pathogens. Induced acid resistance increases the likelihood that a pathogen will survive passage through the stomach. These adaptive systems have also been shown to influence a number of the body's other defense mechanisms. Prior exposure of the pathogens such as enterohemorrhagic *E. coli*, *Shigella*, *Salmonella*, and *L. monocytogenes* to moderately acidic conditions has been shown to increase their ability to survive the conditions that they would be exposed to during their passage through the stomach and upper intestinal tract. More recent research with *Salmonella* and other enteric pathogens suggest that prior exposure of the microorganisms may also directly affect the expression of virulence determinants such as attachment and invasiveness. Thus, accurate assessment of dose-response relations may require consideration of the nature of the food matrix and its effect on the microorganism's pathogenicity.

In addition to direct effects on the pathogenic microorganism, the physical characteristics of the food in which a pathogenic bacterium is transmitted can dramatically influence dose-response relations. For example, anything that either increases stomach pH, decreases the microorganism's exposure to stomach acid, or decreases transit time through the stomach would be expected to decrease the effectiveness of this component of the body's defense against foodborne pathogens. Consumption of highly buffered foods, use of antacids, and achlorohydria (depressed production of gastric acid) would all be expected to decrease the doses needed to elicit infections. Similarly, it has been hypothesized that entrapment of bacterial cells within the fat droplets of emulsified foods (e.g. ice cream) can protect bacterial cells from exposure to stomach acid. The initial rapid transit of liquids when consumed on an empty stomach versus transit time when a solid food is consumed on a full stomach can impact significantly on observed dose-response relations.

2.4. Sources of data

An appreciation of the factors described above is critical to a rigorous consideration of the strengths and weaknesses of the various potential sources of data that can be used to elucidate dose-response relation. An appreciation of these strengths and weaknesses is critical to establishing the uncertainty associated with dose-response models which are based on data derived from various sources and test protocols.

The primary source of dose-response data has been human volunteer feeding studies. Such trials

provide the most direct measure of human response to pathogens and have been the data of choice for quantitative microbial risk assessments. However, these data do have limitations that must be considered when these dose-response relations are going to be used to estimate the susceptibility of the entire population. Volunteers for these studies have been almost exclusively limited to healthy adult males. Information on the susceptibility of higher risk subpopulations or potential gender effects is generally not available. Of necessity, volunteer studies have almost always been limited to foodborne diseases that are not considered life-threatening for the test subjects. Thus, volunteer feeding studies are unlikely to be conducted for diseases that are either life threatening (e.g. enterohemorrhagic *E. coli*) or affect almost exclusively high risk subpopulations (e.g. *L. monocytogenes*). Volunteer studies have often been conducted in conjunction with vaccine trials, which tend to focus on higher dose levels. Typically, there are relatively few test subjects per dose, and because of the small size of the test population, dose levels are used that produce relatively high rates of infection or morbidity. It is usually not possible to evaluate doses that are directly pertinent to the pathogen levels most often associated with human exposures via food. Thus, most dose-response determinations rely on extrapolations of the dose-response relations based on high doses, which can lead to a high degree of uncertainty at the low dose levels.

The primary alternative to human feeding studies is the use of animal models. The successful use of animal models is dependent on a number of factors, not the least of which is the need for a 'conversion factor' that allows the quantitative relations observed in the animal to be correlated with human response to the pathogen. Success is highly dependent on the selection of an appropriate animal model. This can be a significant challenge with many foodborne pathogens. It assumes that the pathogen causes disease by the same mechanism of pathogenicity in both man and animal, that the animal's physiological and immune responses are similar to that of humans, and that quantitative relationships between infectivity, morbidity and mortality are similar for the two species. Further, animal feeding studies have many of the same difficulties as human volunteer studies. For example, most studies are conducted using only healthy animals that are similar in age and weight. In fact, most laboratory animals are so highly inbred that genetic diversity among the animals is negligible. This reduces the variability associated with the testing but brings into question the data's applicability to the general population.

Potentially, epidemiological investigations could be a source for human dose-response information, particularly for outbreaks involving ready-to-eat foods that do support the growth of the pathogenic bacterium. However, to be useful for risk assessments, the investigations would have to be expanded well beyond their current scopes. In addition to detailed information about who became ill, the investigations would also have to acquire information about variety of other factors such as who consumed the food and did not become ill, the amounts of food consumed by both groups, and the frequency and extent of contamination. Regretfully, few epidemiological investigations have been conducted in a manner that provided such data.

As an alternative approach to using epidemiological data to develop dose-response relations for pathogens that are not appropriate for human volunteer feeding studies, Buchanan et al. (1997a) proposed that data on the annual national incidence for a disease could be coupled with food survey data on the frequency and extent of contamination of ready-to-eat food to produce a conservative estimate of the microorganism's dose-response relations. Assuming that all cases of listeriosis were due to a single food, this approach was used to generate a conservative estimate of the dose-response relations for *L. monocytogenes* in high risk populations.

2.5. Empirical modeling of dose response relations

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 often observed. This was traditionally interpreted as indicating that there is a threshold level of pathogenic bacterial cells that must be ingested in order for the microorganism to produce an infection or a disease response in the host. This led to the concept of minimum infectious dose, i.e. the minimum number of bacteria needed to cause disease. There has been substantial effort to define the minimum infectious dose for various

foodborne pathogens. These efforts have typically not been successful. Recently, there has been an ongoing debate concerning the validity of the underlying assumption that such a threshold exists for infectious and toxico–infectious agents. As free-living microorganisms that are capable of reproducing in the intestinal tract, it is difficult to dismiss the potential that under the correct circumstances (e.g. lack of sufficient host defenses), a single cell of an infectious agent could produce an active infection. This is supported by well documented investigations of *E. coli* O157:H7, *S. enteritidis*, and *Shigella* outbreaks where pathogen levels in the incriminated foods were extremely low.

An alternative hypothesis is that if one considers a large enough cross section of the human population, the ingestion of a single pathogenic bacterial cell has a finite possibility of causing an infection, and that this probability increases as the levels of the pathogen increase (Haas, 1983; Rose and Gerba, 1991). For example, it has been estimated that a single *Shigella* cell, a pathogen noted for its high infectivity, has a probability of 0.005 of causing an infection (Crockett et al., 1996). Another way of expressing this concept is that if 1000 people each consumed one *Shigella* spp. cell, five individuals in the group would become infected.

Originally, dose-response relations were largely described using single value estimates of biological endpoints. For example, the simple technique of Reed and Muench (1938) has been used for over 60 years to estimate LD_{50} values, a means of describing the relationship between levels of pathogenic microorganisms and the frequency of mortality. More recently a number of non-threshold mathematical models have been used to describe the entire sigmoidal dose-response curve. Using curve fitting software, it is relatively easy to take experimental data and fit it to one or more of these models. However, it is important to note that all of the models are empirical and cannot be used to infer the underlying physiological basis for pathogenicity.

Two of the more widely used models for fitting dose-response data are the exponential (Table 1, Eq. (1)) and beta-Poisson (Table 1, Eq. (2)) models that were initially introduced by Haas (1983). These have been used by several investigators to describe dose-response relations for a number of different classes of biological agents, including extrapolating to the ingestion of low levels similar to what would be expected in food and water (Haas, 1983; Rose and Gerba, 1991; Crockett et al., 1996; Medema et al., 1996; Buchanan et al., 1997a; Coleman and Marks, 1998). The exponential model assumes that the probability of a cell causing infection is independent of dose, whereas the beta-Poisson assumes that

Table 1
Mathematical models that have been used to empirically describe dose-response data for foodborne pathogenic bacteria

1. Exponential (Haas, 1983):

$$P_i(d) = 1 - \exp(-rd)$$

where: $P_i(d)$ = probability of infection at dose (d)
d = dose (CFU)
r = model parameter specific for each pathogen

2. Beta-Poisson (Haas, 1983):

$$P_i(d) = 1 - (1 + d/\beta)^{-\alpha}$$

where: $P_i(d)$ = probability of infection at dose (d)
d = dose (CFU)
α = model (infectivity) parameter
β = model (shape) parameter

3. Weibull-Gamma (Farber et al., 1996):

$$P_i(d) = 1 - [1 + (d^b)/\beta]^{-\alpha}$$

where: $P_i(d)$ = probability of infection at dose (d)
d = dose (CFU)
b = model (shape) parameter
α = model (infectivity) parameter
β = model (infectivity) parameter

4. Weibull (Krewski and van Ryzin, 1980):

$$P_i(d) = 1 - \exp(-ad^b)$$

where: $P_i(d)$ = probability of infection at dose (d)
d = dose (CFU)
a = model (infectivity) parameter
b = model (shape) parameter

5. Gompertz (Coleman and Marks, 1998):

$$P_i(d) = 1 - \exp[-\exp(a + bf(d))]$$

where: $P_i(d)$ = probability of infection at dose (d)
d = dose (CFU)
a = model (intercept) parameter
b = model (slope) parameter
$f(x)$ = function of dose

infectivity is dose dependent. Both equations are non-threshold sigmoidal functions. The non-threshold character of the equations is more evident when the probability of a response is converted to log values so that log dose versus log response plots are used instead of the more traditional log dose versus response plots (Fig. 1).

The Weibull-gamma model (Table 1, Eq. (3)) has recently begun to be used for dose-response modeling (Farber et al., 1996). This model is based on the Weibull model (Table 1, Eq. (4)) with the assumption that the probability that any individual cell can cause an infection is distributed as a gamma function. This model provides flexibility in that it can take on several different shapes depending on the parameter values selected. Further, several other models, such as the exponential and beta-Poisson models are considered special cases of the Weibull-gamma model.

The Gompertz model (Table 1, Eq. (5)), which has been used extensively in predictive microbiology, has been recently proposed as an effective model for describing dose-response data (Coleman and Marks, 1998).

The above dose-response models have been developed and applied for describing the dose-response relations for infectious and toxico–infectious microorganisms. Alternative models may be required to describe dose-response relations for toxigenic microorganisms. Since these biological agents affect the host via a pre-formed toxin, the extent of disease relates to the levels of toxin ingested by consumers. However, microbial levels are important since toxin production is linked to microbial proliferation. In such cases, the exposure assessment must consider both microbiological and chemical attributes, while the dose-response assessment reverts to consideration of the toxin. This includes a variety of toxin classes such as acute toxicities (e.g. *S. aureus* enterotoxin, *C. botulinum* neurotoxin), chronic toxicities, or carcinogenicity (e.g. aflatoxins). Unlike the infectious and toxico–infectious pathogens, some of these organisms and their toxins display population thresholds that must be reached before there is a host response. For example, it appears that *S. aureus* does not produce sufficient enterotoxin to cause disease until the levels of the microorganism in foods are greater than 10^5 CFU/g.

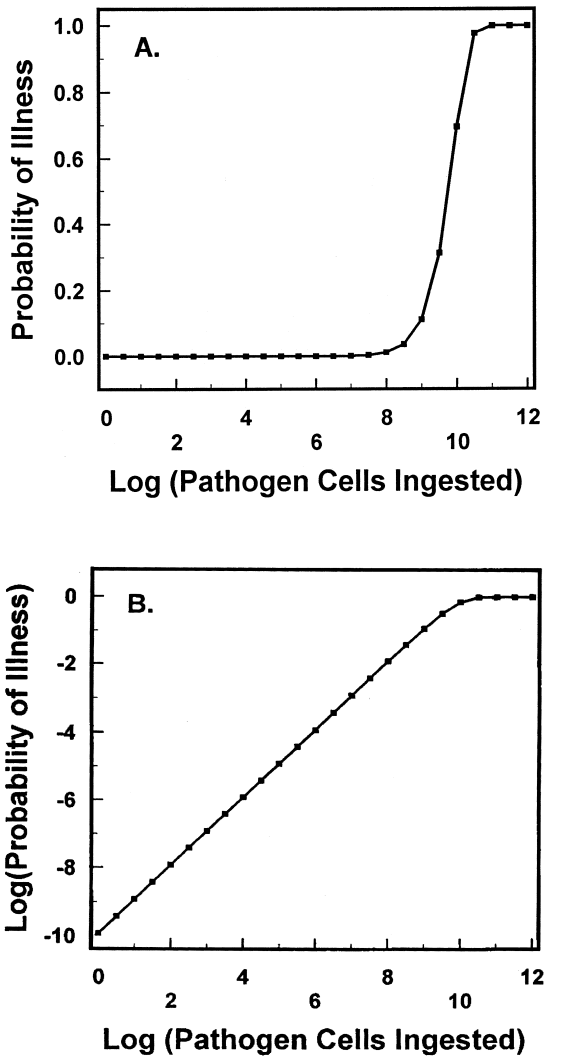

Fig. 1. Example of a dose-response curve generated via an exponential model and plotted as either a log(dose) versus response curve (A) or log(dose) versus log(response) curve (B) (Buchanan et al., 1998b).

2.6. Development of mechanistic dose-response models

Empirical models, such as those described above, are often accurate and highly effective. However, their usefulness is limited by the fact that models of this type should not be extrapolated to consider new conditions or factors. For example, while empirical

models based on human volunteer feeding studies using healthy males may provide excellent dose-response estimates for that group, it would be difficult to directly extrapolate that model to predict the dose-response relations for 80-year-old females. Advances in our understanding of dose-response relations for foodborne pathogens, particularly considering the limitations related to the availability of data pertinent to the entire population, will likely require an alternate, more mechanistic approach to dose-response modeling.

Regretfully, there appear to have been few attempts to develop mechanistic dose-response models for foodborne pathogens. As a means of demonstrating how such a mechanistic model might be developed, we have developed a simple three-compartment dose-response model. The three compartments proposed are (1) gastric acidity barrier, (2) attachment/infectivity, and (3) morbidity/mortality.

2.6.1. Gastric acidity barrier

The first major defense of the body against foodborne pathogens is the acidity of the stomach (Giannella et al., 1973). The key factors influencing the extent of inactivation of ingested pathogens by this barrier are the pH of the stomach, the residence time of the bacteria in the stomach, and the pathogen's inherent acid resistance. Since the inactivation of bacteria due to adverse pH values follows first order kinetics (Buchanan et al., 1997b), the extent of inactivation will also be dependent on the initial numbers of bacterial cells (i.e. dose) ingested. Using an extrapolation of the model for the non-thermal inactivation of enterohemorrhagic *E. coli* (Buchanan et al., 1998a), the effect of pH on the *D*-value (time to a 90% reduction in population) for acid inactivation could be modeled using the equation:

$$\mathrm{Log}(D) = (0.554 \times pH) - 1.429$$

This model was selected since this microorganism is relatively acid resistant. However, the model is based on experimental data for cells that were not pre-adapted to an acidic environment, and thus would be a reasonable, though conservative, model for other pathogenic enteric bacteria.

Using this equation, it is possible to predict how many cells would survive if they remain for a specific time at a specified pH within the stomach

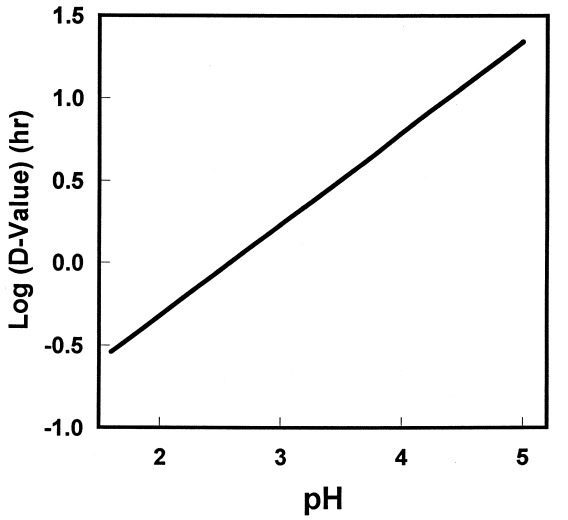

Fig. 2. Model for the effect of pH on the time to achieve a 1−log reduction in the levels of *Escherichia coli* (Buchanan et al., 1998a).

(Fig. 2). However, the emptying of the stomach does not occur at once. Using data for the gastric retention times (Davenport, 1966; Moore et al., 1983) (Fig. 3), it was possible to derive a simple linear model for the clearing of solids from the stomach.

$$\%R = 100.4\ \mathrm{min} + (-0.429 \times T)$$

where:$\%R$ = Percent retention; T = time (min)

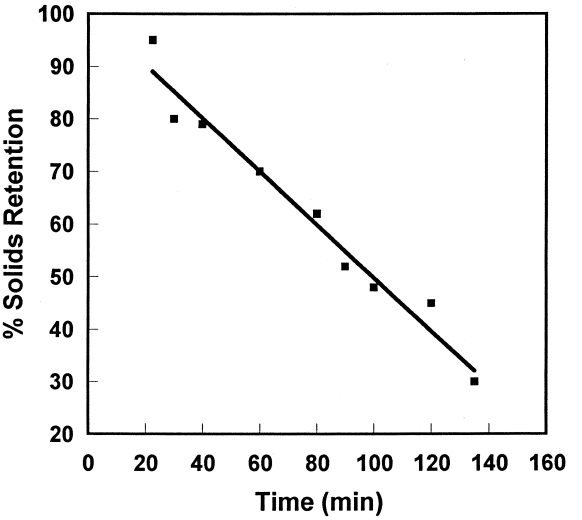

Fig. 3. Model for the emptying time for gastric contents.

Combining these two equations allows calculation of the number of viable cells surviving passage through the stomach and how this is influenced by pH acidity. Assuming that 100 were initially consumed, it becomes evident that at a normal gastric pH of 1.8, only 1–2 viable cells would reach the intestinal tract, whereas raising the pH to 4.0 would allow approximately 50 of the cells to survive (Fig. 4). It is worth noting that these predicted values are consistent with those observed experimentally for enterotoxigenic *E. coli*, *S. typhimurium*, and *Shigella flexneri* (Peterson et al., 1989).

2.6.2. Attachment/infectivity

The next model compartment examines the ability of the bacterial cells that have survived passage through the stomach to attach to and colonize the intestinal epithelium. Without this ability, peristalsis sweeps the pathogen through the intestinal tract, thus preventing infection. The likelihood that any single cell will reach an appropriate site for colonization is dependent on the probability that the pathogen will come in intimate contact with the epithelium and the attachment capability of the organism once it comes in contact with the host cells. Typically, this involves specific locations characteristic of the pathogen. Establishment of an infection site appears to be largely a function of the pathogen's inherent capabilities for attachment and colonization. Most successful pathogens have specific virulence-associated structures (e.g. glycoproteins, glycolipids) on their cell surfaces that interact with receptor sites on epithelial cell surfaces to facilitate attachment. Expression of these virulence-associated cell surface structures are likely to vary among isolates, thus influencing their rate of infectivity. Further, one can speculate that there are a variety of other host and food matrix factors that can influence attachment rates. For example, it has been previously mentioned that acid adaptation of various enteric pathogens subsequently increases receptor mediated attachment to intestinal cells. The rate of peristalsis can also affect intestinal attachment.

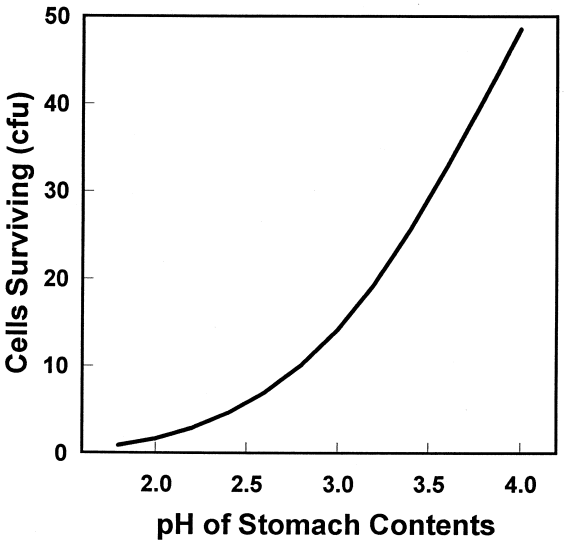

Fig. 4. Predicted effect of pH on the number of *Escherichia coli* cells out of 100 surviving passage through the stomach.

Potentially, the ability of a pathogen to reproduce within the lumen of the intestinal tract and the location of the intestinal attachment sites could also influence the likelihood of attachment. For example, if the attachment site was in the upper sections of the small intestine, the number of pathogen cells that could potentially attach to the infection site would be essentially limited to the number that exited the stomach. However, if the attachment site was further down the intestinal tract, growth of the pathogen in the intestinal contents would increase the likelihood for attachment. This might be counterbalanced somewhat by the fact that the extent of the colonization by an active microflora is much greater in the lower segments of the intestinal tract, thus increasing the likelihood that specific receptor sites would already be occupied by competing microorganisms.

While there is a substantial amount of data available on the interaction of enteric pathogens with epithelial cells, there appear to be few studies that have been conducted in a manner that allow attachment rates in the intestinal environment to be estimated. For the sake of simplicity in the current example, it will be assumed that the attachment site is early in the intestinal tract (i.e. there is no growth of the pathogen after exiting the stomach) and that one cell in 100 actually attaches to the epithelium and establishes an infection. With this ratio, it is possible to use the predicted number of pathogenic cells exiting the stomach to predict the probability of infection (Fig. 5). It is readily apparent that an individual with reduced acid production would have a substantially higher risk of being infected.

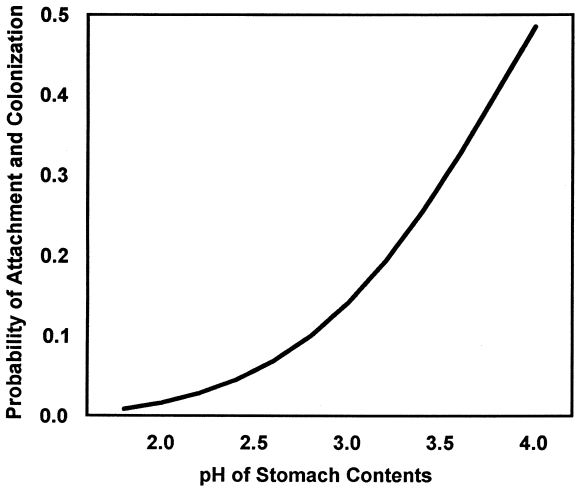

Fig. 5. Predicted effect of pH on the probability of infection after ingestion of 100 cells of *Escherichia coli*.

2.6.3. Morbidity/mortality

The final compartment of the model is the likelihood that an infection progresses to overt symptoms or even death. The primary determinant for this compartment appears to be the capabilities of the host defense systems. In the case of the current example, it has been well established that the immune status of humans declines with age, including antibody production, ability to respond to new antigens, and decreased T-cell and PMN responses (Yehuda and Weksler, 1992; Ernst et al., 1993; Kudlacer et al., 1995; Pahlavani and Richardson, 1996). The specific component of the body's defense system that would have to be modeled would be dependent on the pathogen's mode of pathogenicity.

2.6.4. Scenario assessment

The three-compartment dose-response model described above implies that the rate of infection is primarily dependent on the rate of acid inactivation and the attachment characteristics of the pathogenic microorganism. Once an infection has been established, the extent and severity of disease (i.e. morbidity and mortality) are not necessarily dose-dependent, but instead a function of the virulence characteristics of the pathogen and the immune/health status of the population.

To demonstrate further how such models might be used, a scenario was developed which considers the impact of age on the dose-response relations and the subsequent incidence of salmonellosis. It has long been recognized that attack rates for salmonellosis are substantially higher in the very young and the very old, and these subpopulations account for almost all fatalities (Fig. 6) (Riley et al., 1984; Hargrett-Bean et al., 1988). In developing this scenario, a number of assumptions were made. These assumptions have been purposefully kept simple.

1. One hundred *Salmonella* were ingested by two populations, one consisting of 100 000 individuals over 65 years of age, and 100 000 adults greater than 20 years but less than 65 years.
2. The average rate of achlorohydria (depressed production of gastric acid) in adults ($>$20 years, $<$65 years) is 1 and 30% in the elderly ($>$65 years).
3. The pH of stomach contents is 2.2 for normal adults and 4.0 for individual suffering from achlorohydria
4. Attachment/colonization rates for cells exiting the stomach is one cell per 100.
5. The acid resistance for *Salmonella* is adequately described by that for *E. coli* (Peterson et al., 1989).
6. The percentage of the population that is immune impaired increases after age 65 (Fig. 7), and that immune status influences both the rates of morbidity and mortality (Table 2). (The values for morbidity and mortality were arbitrarily selected

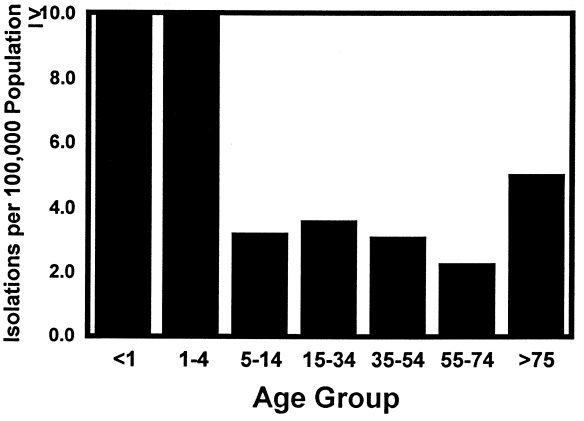

Fig. 6. Incidence of *Salmonella* isolations as a function of age. (Adapted from Riley et al., 1984 and Hargrett-Bean et al., 1988.).

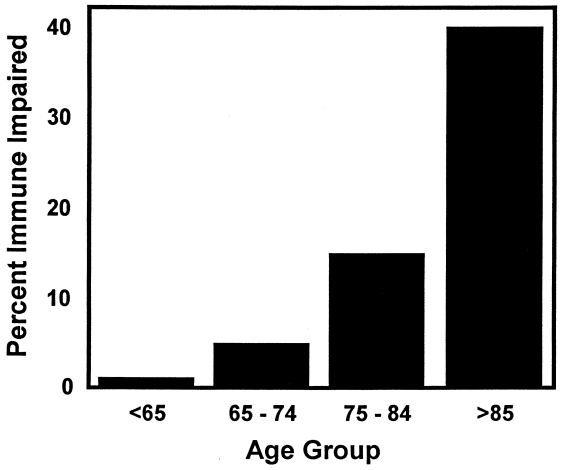

Fig. 7. Assumed effect of age on the frequency of immunoimpaired individuals assumed in risk assessment scenario.

based on reports of outbreaks related to potentially life threatening foodborne infections (e.g. salmonellosis, hemorrhagic colitis, shigellosis)).

7. The distribution of ages for individuals older that 65 years of age is:
   - 59% for 65–74 years
   - 31% for 75–84 years
   - 10% for greater than 85 years

Based on these assumptions, substantially different dose response relations were predicted for the two age groups. First, the number of individuals infected is increased dramatically in the older group due to the increased incidence of achlorohydria (Table 3). Once an infection has been established, the frequency of morbidity and mortality are again higher in the older group due to the increased frequency of immune impairment (Table 4). The increased risk of contracting symptomatic salmonellosis was 5.7 greater for elderly individuals, and the risk of succumbing to the infection was 10-fold greater.

The above scenario provides multiple biological endpoint predictions of the impact of *Salmonella* at a single dose. However, the model can be readily solved for a range of other initial exposure values. This would allow the generation of the more traditional dose-response measurements for infection, morbidity, and mortality.

The current model was selected as a simple example, and will require additional work to increase its accuracy. While this approach requires more effort than the simple fitting of laboratory data to an empirical equation, it provides an assessment that can more closely relate the effect of dose to the biological response being investigated. While the development of mechanistic dose-response models will undoubtedly require a great deal of effort, they will provide some obvious advantages over the empirical models that are used currently.

3. Risk characterization

The final stage in a microbial food safety risk assessment is the development of the risk characterization. Risk characterization is the integration of the exposure and dose-response assessments to provide

Table 2
Assumptions on the effect of immune status on the rates of morbidity and mortality

	% of infected individuals that become symptomatic	% of symptomatic individuals that die
Immune competent	5	2
Immune impaired	10	8

Table 3
Effect of age and achlorohydria on the predicted number of individuals per 100 000 infected after ingestion of 100 cells of *Salmonella*

	Adults (<65 years)	Elderly (>65 years)
Normal acid production	2871	2030
Achlorohydria	459	14 550
Total	3330	16 580

Table 4
Predicted effect of age on the frequency for morbidity and mortality for salmonellosis for a population of 100 000 adults (age between 20 and 65 years) and a corresponding population of elderly (>65 years) based on a simple three compartment mechanistic dose-response model

		<65 years	≥65 total	65–74 years[a]	75–84 years[a]	≥85 years[a]
Number of individuals infected	IC[b]	3297	13 910	8804	4111	995
	II[c]	33	2669	978	1028	663
	Total	3330	16 579	9782	5139	1658
Number of individuals that become symptomatic	IC	165	696	440	206	50
	II	3	267	98	103	66
	Total	168	963	538	309	116
Number of individuals that die	IC	3.3	13.9	8.8	4.1	1.0
	II	0.2	21.3	7.8	8.2	5.3
	Total	3.5	35.2	16.6	12.3	6.3

[a] Results of the >65 years age group subdivided into three ages categories.
[b] Immunocompetent.
[c] Immunoimpaired.

an overall evaluation of the likelihood that the population will suffer adverse effects as a result of the hazard. Mathematically, the exposure assessment serves as the input for the dose-response assessment which when 'solved' provides the risk estimate (i.e. probability of an adverse effect). In addition, the purpose of risk characterization is to communicate the level of confidence that the risk assessors have in their analysis. In addition to the overall interpretation of the results, the risk characterization should recapitulate the impact that critical assumptions and decisions made in developing the exposure and dose-response assessments have on the interpretation of the overall assessment. Since each of the steps or factors that contribute in the production, processing, distribution, preparation, and consumption of foods has its own inherent variability, simulation modeling techniques such as Monte Carlo analysis are increasingly being used to improve the accuracy of quantitative microbial food safety risk assessments. These tools provide a means for considering both the variability of the overall assessment as well as the impact of individual steps. However, the use of these new techniques provides significant challenges in relation to interpreting and communicating the findings generated. Few risk managers or stakeholders are comfortable making decisions on the basis of a probability distribution, though this is really what they have always done. Thus, risk communication is a critical consideration when developing the risk characterization portion of a risk assessment.

A substantial portion of quantitative risk characterizations is devoted to identifying both the confidence intervals associated with the risk estimates and the contribution that individual steps have on the risk. The use of simulation modeling techniques allows this to be performed readily through techniques such as sensitivity analyses. However, care must be exercised in interpreting the results of such analyses. The variability associated with individual steps in a multiple step risk assessment, such as a product/pathogen pathway analysis, can arise from two sources. One is the variability associated with biological systems, food processing technologies, food preparation methods, and human behavior. This variability is inherent to the factors or steps being analyzed. The other type of variability relates to the assumptions which had to be made due to a lack of information. In risk assessments, the former is referred to as variability, while the latter is termed uncertainty. It is critical that the two be differentiated since this will have a tremendous impact on the risk management decisions that emerge as a consequence of the risk assessments. The risk management decisions that need to be reached as a result of having unacceptably high levels of variability are the establishment of control programs, whereas uncertainty is most appropriately handled via the acquisition of

additional information (i.e. research) while the risk is managed through an interim decision. It is worth noting that one of the key benefits of conducting risk assessments is that they quickly identify and help prioritize the research that is needed to address both the elimination of uncertainty and the development of new control or prevention strategies. The analytical differentiation of variability and uncertainty is challenging; however, in most instances a qualitative identification of the relative degree of variability and uncertainty is sufficient for the risk manager to effectively interpret this phase of the risk characterization.

In addition to identifying the likelihood that a hazard will have an adverse impact on the population, risk characterizations should provide an evaluation of the likely severity of that hazard. In general, such severity assessments have been done qualitatively, with a concomitant potential for introducing the risk assessors' biases in relation to the consequences of the disease. An alternative approach that is being examined by different investigators is the use of multiple biological endpoints. By conducting the dose-response assessments so that they examine all pertinent biological endpoints (e.g. rates of infection, morbidity, mortality rates, sequelae), it becomes possible to more objectively evaluate the impact of the disease agent. Then the weight given to consequences of the disease can be more transparently articulated in the risk characterization. The availability of information on the risks of various biological endpoints would be critical if the results of the risk assessment were to be used as part of a subsequent cost-benefit analysis.

An attribute of a successful risk characterization is its transparency. It is only through the successful communication of both the details and conclusions of microbial food safety risk assessments that the validity of individual assessment will be accepted. Such acceptance is critical to the broad adoption of these techniques for the evaluation of microbiological food safety issues. One potential implication of this need to communicate effectively is that more than one version of a risk characterization may be required. While a full disclosure of the mathematical assumptions and scientific data underlying a risk assessment is critical for technical transparency, it can also be a barrier to evaluations by scientists, risk managers, and stakeholders that are not as familiar with the techniques and mathematics. This could be critical since these groups may have information that would drastically change the outcome of the assessment if they were fully cognizant of the scientific and technical issues being addressed. It seems prudent to recommend that two versions of the risk characterization be available; one that contains all of the technical details and a second that interprets those findings in a manner that is readily understood by a broader audience. In this regard, it would be highly advantageous for risk assessment teams to include a least one expert in risk communication when developing risk characterizations.

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