Introduction to Statistical Methods for Quantitative Microbial Risk Assessment

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Introduction

QMRA (Quantitative Microbial Risk Assessment) is a method for assessing risks from microbial agents in a framework that defines statistical probability of an infection fromenvironmental pollution due to sources (water, soil, food, surfaces and hands) (Wijtzes, 2002). Based on the National Academy of Sciences framework for Quantitative Risk Analysis, four-step process for QMRA has been developed by accommodating properties of microorganism, such as physical characteristics, movement in environment, processes of infection, in order to identify where microbes can become a danger based on best measurements about their behavior (Figure 1) (National Academy of Sciences, 1983; Haas et al., 1999).Based on QRMA results,risk management is conducted for decision makers to use when choosing policy and for scientists to see when to run more experiments for better information. In next sections, each of the four steps is described, and (statistical) tools for each step are introduced.

Step 1. Hazard Identification

In order to understand possible transmission routes, it should be determined what microorganisms cause which human illnesses. Diagnostic tools need to be developed to identify hazards and quantify associated infection, incubation time, symptoms in individuals (and communities), severity, duration, and excretion rates. Data sourcesused for hazard identification include clinical data, outbreak investigations, experimental and epidemiological studies.

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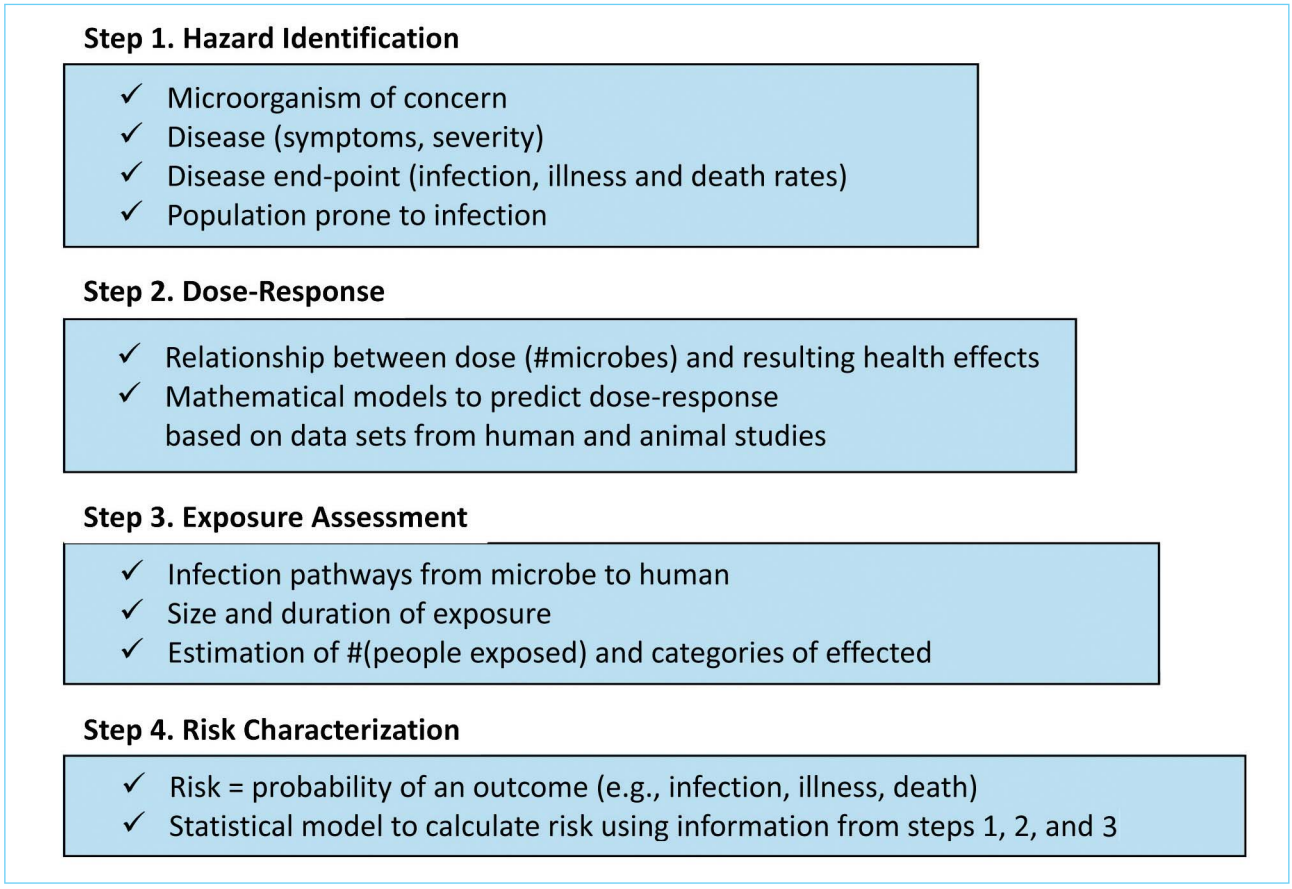

Figure 1. Four-step process for QMRA

There are tools that assist in determining whether a pathogen could be an important hazard in a given food/food process. Examples include semi-quantitative systems, decision trees, expert systems (Notermans and Mead, 1996; Todd and Harwig, 1996; ICMSF, 1996; van Gerwen et al., 1997; van Schothorst, 1997).Decision trees enable experience of others to be shared and can assist in decision making by presenting a structured series of questions relevant to the decision being made. In essence, structured approach of risk assessment offers the same assistance for more complex decision processes.

Step 2. Dose-Response Models

Dose is the number of organisms ingested or inhaled, and response can bean end-point rate (e.g., infection, illness or death rate). The relationship between level of microbial exposure (i.e., dose) and likelihood of health effects (i.e., response) is quantitatively described in a dose-response model. Human and animal feeding studies are used in modeling dose-response relationship. An earlier dose-response model is the exponential model that assumes a constant survival rate (k) for all microorganisms:

$$p=1-\exp(-k\mu)$$

where p is the probability of infection at an event

condition, and μ is the mean dose(Haas 1983). Constant survival rate assumption was removed in later models, including approximate Beta Poisson model:

where p and N_{50} are model specific parameters (Ward et al., 1986).

$$p=1-\left[1+\frac{\mu(2^{1/\alpha}-1)}{N_{50}}\right]^{-\alpha}$$

Step 3. Exposure Assessment

Exposure is the total dose or distribution of doses.To estimates exposure, one need to identifyan affected population; characterize all pathways of exposure and environmental fate; and calculates amount, frequency, length of time and route of exposure for each pathway. The estimated doses vary across pathways, and the estimation requires modeling probability of each pathway and a distribution of dose for a given pathway. Many predictive microbiology models were developed to predict inactivation, survival and growth of microorganisms, by relating kinetics parameters governing behavior of a microorganism to changes in environmental conditions (e.g., temperature). One branch is statistic models, and the other is dynamic models.

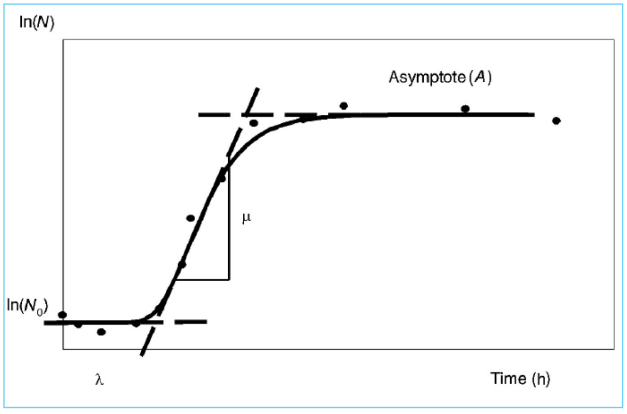

Figure 2. A growth curve of a microorganism

Static Models describe kinetic parameters of microorganisms under fixed (non-changing) environmental conditions. As an example, one temperature value is set, and growth of a microorganism is followed in time (Figure 2). An example would be Gompertz model (Zwietering et al., 1991):

$$\ln\left(\frac{N_t}{N_o}\right)=A\exp\left\{-\exp\left(\frac{\mu_m e}{A}\exp[\lambda-t]+1\right)\right\}$$

where N_t and N_0 are the number of microorganisms at time t and at time 0, respectively; A is the maximum level of microorganisms; μ_m and λ are maximum specific growth rate and lag time at a specific value of temperature.There are many other static models, such as modified Gompertz equation, Baranyi equation, and logistic equation(Baranyi et al., 1993; Gibson et al., 1988; Buchanan et al., 1997). Combinations of linear models for exponential, lag and exponential or lag, exponential and stationary phases have also been used.

Dynamic models describe kinetic parameters of microorganisms under changing environmental conditions. For example, changes in microbiological behavior are monitored against changes in temperature over time. They can be usually a set of differential equations. An example is the first-order exponential growth model with time-delayed growth:

$$\begin{cases} \text{For } t<\lambda: \dfrac{dN}{dt}=0 \\ \text{For } t\geq\lambda: \dfrac{dN}{dt}=\mu N \end{cases}$$

There are three ways to develop predictive models: empirical modeling based on experimental data, mechanistic or deterministic modeling based on a

theoretical understanding of microbiological behavior, and quasi-mechanistic modeling on both. In empirical modeling, literature study should be carried out to identify available data and existing relevant models. Experimental design can be very important (Davies, 1993; McMeekin et al., 1993; Ratkowsky, 1993). For instance, gguidelines for data collection and storage from experiments (Kilsby and Walker, 1990; Walker and Jones, 1993). Measuring microbial numbers over time produces growth curves, and parameters of growth curve can be determined through a process of statistical regression called fitting. Finally models are validated by training, testing and validation data. However, models built from empirical modeling provide no secure basis for extrapolation outside range of experimental data.Extrapolation without validation may still be dangerous because mechanism itself may change, or prediction errors may become very large. In mechanistic modeling, it can be justified whenever a basic understanding of system is essential to progress or when state of the art is sufficiently advanced to make a useful mechanistic model easily available(Box et al., 1978). Quasi-mechanistic modeling has all indicated linkages between putative mechanisms in deterministic modeling and observations of growth responses used in empirical modeling.

Tools for developing predictive models include general statistical packages such as SAS and SPSS. These packages are useful in model development in such areas as experimental design, data handling, and establishing prediction and confidence intervals. Although there is no integral package dedicated to whole process of modeling microbial behavior, there are a number of packages which are helpful in model design. A widely used tool for performing probabilistic modeling is @RISK, which is a risk analysis and simulation add-in for Microsoft Excel. It uses Monte Carlo simulation to allow an assessment to be made of all possible outcomes by recalculating a spreadsheet hundreds or even thousands of times, each time selecting random numbers from distributions that were entered. Results are distributions of possible outcomes and probabilities of getting those results. There are off-the-shelf models, such as Pathogen Modeling Program (US Department of Agriculture), *Food MicroModel*(UK government), and *FORECAST* (Campden and Chorleywood Food Research Association). Last, decision support systems are ready to use software packages for probabilistic microbial risk modeling do not exist yet. For examples, MIDAS system (joint Unilever-Bestfood Research Laboratories), Food Design Support System (Wageningen Agricultural University) and SIEFE (Stepwise and Interactive Evaluation of Food safety by an Expert system) program (Wageningen Agricultural University).

Step 4. Risk Characterization

Based on a statistical model with inputs, risk can be characterized by integrating dose-response analysis and exposure assessment. All inputs are likely to vary, and hence are random variables. A major tool for describing variability is probability density function (PDF). PDF can be estimated by applying maximum likelihood method to experimental data. Examples for statistical models include Monte Carlo simulation, Latin Hypercube simulation, Wells-Riley model, and sequential box

model.

When overall probability of infection or magnitude of risk is estimated by integrating dose-response analysis and exposure assessment, one may consider uncertainty and variability of estimated doses across exposure pathways and multiple exposures. First, the overall probability of infection can be estimated under all pathways for single exposure.Single exposure risk is:

$$p_{\text{single}} = \pi_0 p_0 + \sum_{i=1}^{n} \pi_i p_i$$

where n = total number of pathways, π_0 = probability of baseline/nominal pathway occurring, p_0 = probability of infection under baseline/nominal pathway, π_i = probability of ith pathway occurring, and p_i = probability of infection under ith pathway. Second, the overall probability of infection from multiple exposures can be estimated by assuming that each exposure event is independent and multiple exposureshave same exposure scenario:

$$p_{\text{multiple}} = 1-(1-p_{\text{single}})^{M}$$

where p_{single} = overall probability of infection from a single exposure and M = total number of exposures. Thus the expected number of infections is $Mp_{single.}$ Third, the overall probability of infection under multiple exposure risk with different exposure scenarios is:

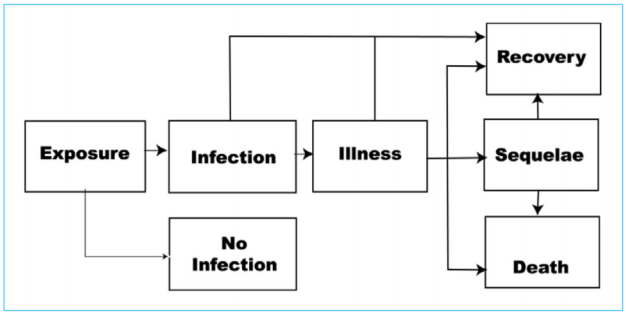

Figure 3. Disease outcomes of exposure

$$p_{\text{multiple}} = 1-\prod_{l=1}^{L} (1-p_{\text{single},l})^{M_l}$$

where $P_{single,l}$ = overall probability of infection under lth scenario, L = total number of different single infection rate, and M_l = total number of exposures under lth scenario.

For exposures, there are many possible disease outcomes (Figure 3). Based on characterization of disease development process with multiple disease outcomes, general health burden on a community per case of illness can be described by disability adjusted life years (DALY):

$$DALY = P(\text{illness}|\text{infection}) \sum_{j=\text{i}}^{J} P(outcome_j|\text{illness}) D_j S_j$$

where P(illness|infection) = probability of illness given infection, $P(outcome_j|\text{illness})$ = probability of outcome j given illness, D_j = duration (years) of outcome j,andS_j = severity weighting for outcome j. An advantage of using DALY is that it reflects not only the effects of acute end-points but also likelihood and severity of more serious disease outcomes (WHO GDWQ, 2004; Havelaar et al., 2000).

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