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Statistical models of health risk due to microbial contamination of foods

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Between 6 million and 33 million cases of food-related illness are estimated to occur in the United States each year, with about 5000 episodes resulting in death. Growing concerns about the safety of food prompted the National Food Safety Initiative of 1997, the goal of which is to reduce the incidence of illness caused by food-borne pathogens. A key component of the food safety initiative is the improvement of farm-to-table risk assessment capabilities, including the development of improved dose-response models for estimating risk. When sufficient data are available, allowable contamination levels of specific micro-organisms in food are established using dose-response models to predict risk at very low doses based on experimental data at much higher doses. This necessitates having reliable models for setting allowable exposures to food-borne pathogens. While only limited data on relatively few micro-organisms that occur in food are available at present for dose-response modeling and risk estimation, still none of the two-parameter models proposed so far, including the popular Beta-Poisson (BP) model, appears to be completely satisfactory for describing and fitting all of the present data (Holcomb *et al*., 1999). The Weibull–Gamma (WG) model is the only three-parameter model that has been proposed to date. In this paper, new competitive three-parameter models are derived, using a formulation that can be parameterized to represent statistical variation with respect to the dose of micro-organism received by the host and the host's susceptibility to infection. Parameters of the models are estimated using the maximum likelihood method. Experimental data on several common microbial contaminants in food are used to illustrate the methodology.

Keywords: dose-response, food-borne pathogen, food safety initiative, host susceptibility, low-dose extrapolation, risk assessment

1. Introduction

Many types of micro-organisms can cause infection and disease when transmitted to individuals through the food supply. Food-borne pathogens are reported to cause between

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6 million and 33 million illnesses each year in the United States, with approximately 5000 resulting in death. The National Food Safety Initiative of 1997 (FDA, USDA, EPA, CDC, 1997) was the result of increasing concern about the safety of the nation's food supply. Its goal is to reduce the incidence of illness caused by food-borne pathogens by improving the farm-to-table risk assessment process.

In order to control diseases caused by microbial contaminants in food, it is essential to be able to assess their dose-response relationships as accurately as possible (Marks *et al.*, 1998). However, definitive dose-response data on humans at levels of contamination likely to occur in practice are scarce to nonexistent. Hence, when sufficient animal or human data at high doses are available to allow dose-response modeling, allowable contamination levels of specific micro-organisms in food are established using dose-response models to predict risk at very low doses based on the high-dose data.

Unlike the case of chemical risk assessment, at present there do not exist generally accepted practices for estimating microbial risk, although important work has been done on the use of predictive models in risk assessment for food-borne pathogens (Buchanan and Whiting, 1996; Teunis *et al.*, 1996; Marks *et al.*, 1998; Haas *et al.*, 1999). To date, the most common statistical dose-response models have been the simple exponential, the lognormal and the Beta-Poisson (Haas, 1983). These models have a degree of biological basis, having been derived from varying assumptions regarding micro-organism infectivity and host susceptibility. However, these one- and two-parameter models may not be flexible enough to fit dose-response data on all types of micro-organisms, especially in cases where there are data at multiple dose levels. The three-parameter Weibull-Gamma (WG) model has been proposed recently (Farber *et al.*, 1996) as a model that offers more flexibility. In this paper, a general approach to the development of microbial dose-response models is described. The procedure incorporates both dose- and host-variability into expressions for the probability of infection. Existing one-, two- and three-parameter models are derived as special cases, and new three-parameter models are proposed. The models are fitted to 22 existing data sets on a variety of micro-organisms. Four of these data sets were analyzed previously by Holcomb *et al.* (1999) using six different dose-response models, of which four models are included in this study.

2. Development of dose-response models

2.1 *General approach*

Variability in host susceptibility is a recognized characteristic that must be considered in the development of dose-response models for microbial infection (Haas, 1983). Let $p(t;j)$ be the probability that an individual with infectivity parameter t becomes infected after short-term exposure to j micro-organisms and let $f(\theta;t)$ be the probability density of t in the population of interest. The expected proportion of individuals in the population becoming infected after exposure to j micro-organisms is thus (Pinsky, 2000)

$$r(\theta;j) = \int p(t;j)f(\theta;t)dt. \tag{1}$$

Here $f(\theta;t)$ is taken to represent only variation in host susceptibility, but it might also be considered to account for variation in the virulence of a micro-organism (Haas, 1983).

There are two opposing views regarding the process of microbial infection of a host, stochastic and deterministic. The stochastic view assumes that the micro-organisms act independently and that a single micro-organism is capable of causing infection in an individual (Armitage *et al*., 1965), i.e., there is no threshold. The stochastic view appears to apply to infectious food-borne pathogens such as *Salmonella spp*., *Shigella spp*., *Escherichia coli* O157:H7 and *Listeria monocytogenes* (Buchanan and Whiting, 1996). If the infectivity parameter t represents the constant probability that a single micro-organism will cause infection, then the stochastic, nonthreshold model gives

$$p(t,j) = 1 - (1-t)^j. \tag{2}$$

Common choices for $f(\theta;t)$ to accompany $p(t;j)$ defined in (2) are the beta distribution,

$$f(\alpha,\beta;t) = \frac{\Gamma(\alpha+\beta)}{\Gamma(\alpha)\Gamma(\beta)} t^{\alpha-1}(1-t)^{\beta-1}, \quad 0<t<1, \quad \alpha>0, \quad \beta>0,$$
$$\theta = (\alpha,\beta), \tag{3}$$

and the point-mass distribution,

$$f(\theta;t) = I_{[t=\theta]}. \tag{4}$$

With the beta and point-mass distributions for $f(\theta;t)$, and with $p(t;j)$ as given in (2), the expected proportion of infected individuals become

$$r(\alpha,\beta;j) = 1 - \frac{\Gamma(\alpha+\beta)\Gamma(\beta+j)}{\Gamma(\beta)\Gamma(\alpha+\beta+j)} \tag{5}$$

and

$$r(\theta;j) = 1 - (1-\theta)^j, \tag{6}$$

respectively.

The deterministic view of the process of microbial infection assumes that micro-organisms act collectively, and that a threshold dose is necessary to cause infection in an individual. It appears that the deterministic view might apply to toxic food-borne micro-organisms such as *Staphylococcus aureus* and *Clostridium botulinum* (Buchanan and Whiting, 1996), in that a certain number of micro-organisms might be required to produce enough toxin to elicit a response. The deterministic view essentially means that each individual in a population has a threshold tolerance, t, to infection by the micro-organisms, which leads to

$$p(t;j) = I_{[t,\infty]}(j) = \begin{cases} 1, & \text{if} \quad t \le j \\ 0, & \text{otherwise.} \end{cases} \tag{7}$$

Common choices for $f(\theta;t)$ to accompany $p(t;j)$ defined in (7) are the lognormal (LN) distribution and the log-logistic (LL) distribution. With $p(t;j)$ given by (7), $r(\theta;j)$ becomes the cumulative distribution function of the corresponding probability density, $f(\theta;t)$, of t. Thus, for the lognormal, the expected proportion of infected individuals is

$$r(\mu,\sigma;j) = \frac{1}{\sqrt{2\pi}}\int_{-\infty}^{(\log j-\mu)/\sigma} \exp(-t^2/2)dt, \quad -\infty < \mu < \infty, \quad 0 < \sigma,$$
$$\theta = (\mu,\sigma), \tag{8}$$

and for the log-logistic it is

$$r(\mu,\sigma;j) = \frac{1}{1+\exp[-(\log j-\mu)/\sigma]}, \quad -\infty < \mu < \infty, \quad 0 < \sigma, \quad \theta = (\mu,\sigma). \tag{9}$$

Let i index the dose groups in a human trial or an animal experiment. Let d_i denote the intended dose to the subjects in group i. Because of the nature of working with micro-organisms, the dose that experimental subjects actually receive is a random variable with a nominal value of d_i. Let $g(d_i)$ denote a probability mass function that governs the actual dose received by subjects in the i-th group, and assume that doses received by individual subjects are independent of each other. Assuming that micro-organisms are distributed randomly in the dose vehicle, the Poisson distribution has been proposed as a reasonable distribution for the actual dose received (Worcester, 1954). With d_i representing the average number of micro-organisms administered to the i-th dose group, the Poisson probability that an individual receives dose j is

$$g(d_i;j) = \frac{d_i^j \exp(-d_i)}{j!}. \tag{10}$$

If $g(d;.)$ is the exposure distribution for a particular micro-organism, then the expected proportion infected in the i-th dose group after exposure at a nominal level of d_i is (Pinsky, 2000)

$$P(d_i;\theta) = \sum_{j=1}^{\infty} g(d_i;j)\int_{-\infty}^{\infty} p(t;j)f(\theta;t)dt. \tag{11}$$

Expression (11) provides a general formula for deriving dose-response models for the probability of microbial infection. It includes components to account for variability in the amount of dose received and the level of individual susceptibility to infection.

2.2 *Existing models*

Specific choices for g, p, and f in (11) give rise to a number of existing models for $P(d_i;\theta)$. If g is Poisson with probability mass function given by (10), f is the point-mass distribution given by (4) and p is the nonthreshold susceptibility probability given by (2), then the probability of infection is exponential (cf, Haas, 1983),

$$P(d_i;\theta) = 1 - \exp(-d_i\theta). \tag{12}$$

The exponential model appears to be the only one-parameter dose-response model that has been proposed for microbial risk assessment.

Various existing two-parameter models may also be derived using (11). If g is Poisson as in (10), f is beta as in (3) and p is nonthreshold as in (2), then the so-called Beta-Poisson (BP) model (Furumoto and Mickey, 1967; Haas, 1983) is obtained as

$$P(d_i;\alpha,\beta)\approx 1-(1+d_i/\beta)^{-\alpha}\quad(\beta\gg\alpha). \tag{13}$$

If g is Poisson with mean $\exp(\alpha+\beta\log d_i)$, f is the point-mass distribution $I_{[t=1]}$ and p is the nonthreshold model in (2), then the distribution of the smallest extreme-value (EV) (Marks *et al*., 1998) is obtained as

$$P(d_i;\alpha,\beta)=1-\exp[-\exp(\alpha+\beta\log d_i)],\quad -\infty<\alpha<\infty,\quad \beta>0. \tag{14}$$

If g is the point-mass distribution, i.e., $g(d_i,j)=I_{[j=di]}$, f is the lognormal density with cumulative distribution given by (8) and p is the threshold model in (7), then the LN distribution is recovered as

$$P(d_i;\mu,\sigma)=\frac{1}{\sqrt{2\pi}}\int_{-\infty}^{(\log d_i-\mu)/\sigma}\exp(-t^2/2)dt,\quad -\infty<\mu<\infty,\quad 0<\sigma. \tag{15}$$

Similarly, if g is the point-mass distribution, $g(d_i,j)=I_{[j=di]}$, f is the LL density with cumulative distribution given by (9) and p is the threshold model in (7), then the LL distribution is recovered as

$$P(d_i;\mu,\sigma)=\frac{1}{1+\exp[-(\log d_i-\mu)/\sigma]},\quad -\infty<\mu<\infty,\quad 0<\sigma. \tag{16}$$

These two-parameter models, along with the one-parameter exponential model, have been the most prominent models in microbial risk assessment, especially the two-parameter Beta-Poisson.

The only three-parameter model that has gained some acceptance is the so-called WG model (Farber *et al*., 1996), which may be obtained from (11) with g being Poisson with mean d_i^{γ}, p being nonthreshold as in (2) and f being gamma (α,β), i.e.,

$$f(\alpha,\beta;t)=\frac{1}{\Gamma(\alpha)}\beta^{\alpha}t^{\alpha-1}\exp(-\beta t),\quad \alpha>0,\quad \beta>0. \tag{17}$$

The resulting dose-response model is

$$P(d_i;\alpha,\beta,\gamma)=1-[1+d_i^{\gamma}/\beta]^{-\alpha},\quad \alpha>0,\quad \beta>0,\quad \gamma>0. \tag{18}$$

The WG model is so-named because $h(d_i;t)$ is Weibull and $f(\theta;t)$ is gamma, where $h(d_i;t)$ is defined in general by

$$h(d_i;t)=\sum_{j=1}^{\infty}g(d_i;j)p(t;j), \tag{19}$$

so that

$$P(d_i;\theta)=\int_{-\infty}^{\infty}h(d_i;t)f(\theta;t)dt. \tag{20}$$

With this naming convention, it seems that the Beta-Poisson should be called the Exponential-Beta model, because for the Beta-Poisson $h(d_i;t)$ is exponential and $f(\theta;t)$ is beta. However, this model has been called the Beta-Poisson since its initial derivation, and thus it would be confusing to change the name after so long a time. Interestingly, the WG model contains both the BP model $(\gamma=1)$ and the LL model $(\alpha=1,\beta=e^{\mu/\sigma},\gamma=1/\sigma)$

as special cases. This is particularly noteworthy given that the individual infectivity probability, p, is a nonthreshold probability in the Beta-Poisson model (just as in the parent WG model) but a threshold probability in the LL model.

There has been some debate as to whether some micro-organisms might require the presence of a colony of cells in order to induce infection even in the most sensitive individual. If so, then a ''population'' threshold would exist. A BP model with a population threshold was considered by Marks *et al*. (1998). This model may be expressed as

$$P(d_i;\alpha,\beta,\tau) = 1 - [1 + (d_i - \tau)/\beta]^{-\alpha}, \tag{21}$$

where $\tau > 0$ represents the threshold parameter.

2.3 *New models*

It is of interest to develop other three-parameter dose-response models for microbial risk assessment as competitors of the WG model. Several of these are now derived using (11).

Let g be Poisson with mean $\alpha + \beta d_i (\alpha > 0, \beta > 0)$, p be the nonthreshold infectivity probability in (2) and f be exponential with parameter $\gamma > 0$. Then

$$P(d_i;\alpha,\beta,\gamma) = 1 - (1 + \alpha/\gamma + \beta d_i/\gamma)^{-1}. \tag{22}$$

Following the naming scheme for the WG model, this model might be called the exponential-exponential (EE) model, because both $h(d_i;t)$ and $f(t;\theta)$ are exponential.

Next let g be Poisson with mean d_i, p be the nonthreshold probability in (2) and f be the gamma density with parameters $\alpha > 0, \beta > 0, \tau > 0$, where τ is a location parameter. Then

$$P(d_i;\alpha,\beta,\tau) = 1 - \frac{\exp(-\tau d_i)}{(1 + d_i/\beta)^{\alpha}}. \tag{23}$$

Because $h(d_i;t)$ is exponential and $f(t;\theta)$ is gamma, the dose-response model in (23) might be termed the exponential-gamma (EG) model. Note that if $\tau = 0$, this model reduces to the BP model.

If g is Poisson with mean d_i^{γ}, p is given by (2) and f is exponential with parameters β and τ, where τ is a location parameter, then

$$P(d_i;\beta,\gamma,\tau) = 1 - \frac{\exp(-\tau d_i^{\gamma})}{(1 + d_i^{\gamma}/\beta)}. \tag{24}$$

With $h(d_i;t)$ being Weibull and $f(t;\theta)$ being exponential, this model might be termed the Weibull-exponential (WE) model. Setting $\tau = 0$ in (24) gives the LL model.

To get a model with a population threshold dose, one can take g to be the indicator function $I_{[j=di]}$, p to be the threshold infectivity parameter $I_{[t,\infty]}(j)$ and f to be Weibull (α, β, γ). The result is a shifted Weibull (SW) dose-response model,

$$P(d_i;\alpha,\beta,\gamma) = \begin{cases} 1 - \exp\{-[(d_i - \alpha)/\beta]^{\gamma}\}, & d_i \geq \alpha, \\ 0, & 0 < d_i < \alpha. \end{cases} \tag{25}$$

The parameter α in (25) represents a population dose threshold.

3. Fitting dose-response models to data

Let there be I independent dose groups $(i = 1, 2, \ldots, I)$ with n_i independent subjects per group. Let X_i denote the number of infected subjects in dose group i, with observed value x_i. Assuming that the X_i are distributed Binomial $(n_i, P(d_i; \theta))$, the likelihood function can be expressed as

$$L(\theta) = \prod_{i=1}^{I} \binom{n_i}{x_i} P(d_i; \theta)^{x_i} [1 - P(d_i; \theta)]^{n_i - x_i}. \tag{26}$$

Maximum likelihood estimation based on the likelihood function in (26) was used to fit all two- and three-parameter models described by expressions (13)–(16), (18) and (22)–(25) in Sections 2.2 and 2.3 to 22 sets of real data. Goodness-of-fit was tested by comparing each fitted model's loglikelihood value to that of the saturated model, i.e., the model giving the observed probabilities x_i/n_i for each group. The significance of twice the difference in loglikelihoods was assessed by comparison to $\chi^2(I - \dim(\theta))$, with $\dim(\theta)$ being the dimension of the parameter vector θ. Dose groups with observed proportions of 0 or 1 did not contribute to the loglikelihood for the saturated model, according to the customary practice of taking $0 \cdot \log(0)$ to be zero, but they did contribute to the loglikelihood for the estimated model if the corresponding estimates of $P(d; \theta)$ were between 0 and 1.

The 22 data sets were extracted from the paper by Teunis *et al*. (1996) on dose-response relationships in human volunteers exposed to a variety of gastro-intestinal pathogens. The pathogens (micro-organisms) are listed in Table 1 along with summary information about doses and numbers of subjects. The detailed dose-response data used for modeling are available from the first author (rkodell@nctr.fda.gov). A data set from Teunis *et al*. (1996) was excluded from this study if it did not contain at least one observed response other than 0 or 100%, if the dose-response was extremely flat, or if some dose levels were expressed as fractional numbers of micro-organisms. It should be noted that some of the *Salmonella* data sets include individuals that were given doses at earlier times. The responses in data sets 16, 18 and 20 represent illness rates, while all other data sets reflect infection rates. The implications of basing a risk assessment on infection as opposed to actual illness are discussed by Teunis *et al*. (1999).

Table 2 contains the p-values of the goodness-of-fit tests from the maximum likelihood fitting of each of the models to the micro-organism dose-response data summarized in Table 1. Values less than 0.05 are given in bold type. Data sets numbered 11, 12, 14, 19 and 22 had only three dose groups, so that only two-parameter models were fitted, leaving one degree-of-freedom for testing goodness-of-fit. For all of these data sets, all of the two-parameter models gave adequate fits.

For one data set (*Echovirus* 12, data set 4) none of the models fit acceptably at first. Inspection of the data revealed that the responses at the two highest doses were below those at the two preceding doses. A strong dose-response relationship is apparent through the five doses preceding the two highest doses. It seems unlikely that any reasonable model would be able to produce an ''acceptable'' fit to the full data set. In practice, for making predictions in the low-dose range, one normally discards extreme high doses that give data inversions, and then fits a model to the remaining doses. Such was done in this exercise, giving the p-values that appear in Table 2.

Data sets 3 (*Entamoeba coli*) and 16 (*Salmonella typhi*) were troublesome to most of the

Table 1. Summary information on food-borne microbial pathogens (from Teunis *et al*., 1996).

	Microorganism	*Number of Doses*	*Dose Range*	*Number of Subjects/Dose*
Pathogenic protozoans				
1	*Giardia lamblia*	8	$1–10^6$	2–20
2	*Cryptosporidium parvum*	8	$30 - 1.0 \times 10^6$	1–8
3	*Entamoeba coli*	5	$1 - 10^4$	2–6
Viruses				
4	*Echovirus* 12	4	$3.3 \times 10^2 - 1.0 \times 10^4$	3–50
5	*Poliovirus* type 1 in infants	12	$7 - 280$	1–6
Bacteria				
6	*Campylobacter jejuni*	6	$8 \times 10^2–1.0 \times 10^8$	5–19
7	*Salmonella meleagridis* I	11	$1.2 \times 10^4–5.0 \times 10^7$	5–6
8	*Salmonella meleagridis* III	4	$1.6 \times 10^5–1.0 \times 10^7$	6
9	*Salmonella anatum* I	8	$1.2 \times 10^4–8.6 \times 10^6$	5–6
10	*Salmonella anatum* II	8	$8.9 \times 10^4–6.7 \times 10^7$	6–8
11	*Salmonella anatum* III	3	$1.6 \times 10^5–4.7 \times 10^6$	6
12	*Salmonella newport*	3	$1.5 \times 10^5–1.4 \times 10^6$	6–8
13	*Salmonella derby*	5	$1.4 \times 10^5–1.5 \times 10^7$	6
14	*Salmonella bereilly*	3	$1.3 \times 10^5–1.7 \times 10^6$	6
15	*Salmonella pullorum* IV	5	$1.9 \times 10^6–4.0 \times 10^9$	6
16	*Salmonella typhi*	5	$10^3–10^9$	9–116
17	*Plesiomonas shigelloides*	5	$1 \times 10^3–4 \times 10^9$	3–7
18	*Shigella flexneri* 2	5	$10^4–10^8$	4–19
19	*Shigella paradysenteriae*	3	$10^8–10^{10}$	4–8
20	*Shigella dysenteriae* 1 M31	4	$10^1–10^4$	4–10
21	*Vibrio cholerae* Inaba 569B without pH buffer	7	$10^4–10^{11}$	1–4
22	*Vibrio cholerae* Inaba 569B without pH buffer	3	$10^4–10^8$	2–52

models. Set 3 had a response inversion at the second highest dose level. Nevertheless, all models fit set 3 adequately (5% level). Set 16 showed no apparent problems, but the BP and EE models could not fit those data adequately. This data set provides an example of utilizing a three-parameter model when a certain two-parameter model does not fit adequately. Specifically, the three-parameter WG model was able to fit the data, although its two-parameter BP submodel could not. Also, the three-parameter EG (submodel BP) had an acceptable fit. Instead of comparing goodness-of-fit p-values between the two- and three-parameter models (Table 2), one could use a likelihood-ratio testing strategy to determine if the more general three-parameter model provides a statistically superior fit to a two-parameter submodel. The likelihood-ratio test comparing the WG to the BP gives $\chi^2(1) = 3.88$ with $p < 0.049$. For comparing the EG to the BP, the likelihood-ratio test gives $\chi^2(1) = 5.90$ with $p < 0.016$. Hence, the likelihood-ratio tests and the goodness-of-fit p-values give essentially the same results, suggesting the utility of a three-parameter model when a popular two-parameter submodel of the three-parameter model is inadequate to describe the data.

Table 2. Goodness-of-fit *p*-values for dose-response models fitted to 22 data sets (Teunis *et al*., 1996).

No.	*BP*	*LN*	*LL*	*EV*	*WG*	*EE*	*EG*	*WE*	*SW*
1	0.211	0.203	0.194	0.217	0.139	0.123	0.137	0.141	0.164
2	0.999	0.997	0.996	1.000	0.998	0.979	0.996	0.999	0.999
3	0.155	0.152	0.149	0.155	0.074	0.071	0.125	0.073	0.086
4	0.199	0.241	0.203	0.405	0.189	0.093	0.413	0.200	0.200
5	0.154	0.395	0.380	0.436	0.341	0.051	0.109	0.348	0.348
6	0.659	0.679	0.668	0.693	0.499	0.346	0.696	0.526	0.532
7	0.578	0.588	0.596	0.557	0.494	0.420	0.500	0.496	0.463
8	0.317	0.275	0.311	0.190	0.155	0.127	0.188	0.127	0.148
9	0.259	0.351	0.329	0.419	0.301	0.360	0.392	0.302	0.303
10	0.211	0.245	0.225	0.271	0.181	0.246	0.318	0.182	0.182
11	0.848	0.999	0.999	0.760	*	*	*	*	*
12	0.687	0.545	0.468	0.681	*	*	*	*	*
13	0.859	0.860	0.860	0.849	0.687	0.674	0.689	0.687	0.701
14	0.191	0.191	0.191	0.190	*	*	*	*	*
15	0.566	0.998	0.999	0.999	0.941	0.122	0.365	0.953	0.999
16	**0.035**	0.144	0.093	0.191	0.093	**0.007**	0.262	0.093	0.341
17	0.442	0.320	0.309	0.298	0.290	0.101	0.263	0.166	0.391
18	0.329	0.286	0.290	0.274	0.181	0.088	0.180	0.153	0.226
19	0.606	0.999	0.997	0.638	*	*	*	*	*
20	0.984	0.919	0.906	0.773	0.860	0.216	0.882	0.660	0.759
21	0.883	0.893	0.859	0.825	0.881	0.719	0.830	0.754	0.962
22	0.699	0.722	0.707	0.737	*	*	*	*	*

Of the two-parameter models, the LN, LL and EV were able to fit all 22 data sets based on a 5% significance criterion. Of the three-parameter models, the WG, WE, EG and SW models all were able to fit (5% level) all data sets that had enough data to allow the fitting of a three-parameter model. The three-parameter EE model does not appear to offer any improvement over the two-parameter models. However, the WE, EG and SW models appear competitive with the established WG model for these data sets.

In addition to comparing the fits of the various models, it is of interest to compare estimates of doses corresponding to specific levels of risk. Table 3 provides maximum likelihood estimates of the dose corresponding to a risk level of 0.01 for each of the models. These estimated dose values have been rounded to integers, because integers represent the natural dose metric for micro-organisms. (In an actual risk assessment, it might be more appropriate to truncate than to round non-integer values.) As can be seen in Table 3, for some data sets the estimated doses are quite comparable, while for others, there is large disparity among the estimates. Hence, even at a risk level of 0.01, there is potential to reach vastly different conclusions regarding the toxicity of a particular micro-organism, depending on the specific model used to describe the dose-response data. In particular, the SW model gave estimates that sometimes differed greatly from those of the other models. Where these were judged to be unrealistically large by the authors, they have not been reported, and are instead indicated by an asterisk in Table 3. Even some SW estimates that are judged to be reasonable in terms of the data (e.g., data set 7) are much larger than those based on the other models. As might be expected, it appears that

Table 3. Maximum likelihood estimates of the dose corresponding to a risk of 0.01 for 22 data sets.

No.	*BP*	*LN*	*LL*	*EV*	*WG*	*EE*	*EG*	*WE*	*SW*
1	0	1	0	0	1	0	1	0	0
2	2	5	3	1	1	1	2	1	1
3	0	0	0	0	0	0	0	0	1
4	6	1	0	0	40	11	5	0	329
5	1	24	20	13	18	1	1	14	15
6	1	0	0	0	0	0	0	0	*
7	135	9	6	0	8	0	105	4	4500
8	5040	1×10^4	5458	121	1×10^4	5584	5602	7071	1×10^5
9	220	3	14	0	1	0	0	1	101
10	68	0	0	0	0	0	0	0	10
11	3183	1×10^5	1×10^5	6390	*	*	*	*	*
12	2508	2×10^4	1×10^4	1300	*	*	*	*	*
13	0	0	0	0	0	0	0	0	*
14	0	0	0	0	*	*	*	*	*
15	1×10^7	7×10^8	9×10^8	2×10^8	1×10^8	1×10^7	2×10^8	5×10^8	
16	1481	79	2	0	0	0	574	0	1000
17	26	0	0	0	928	0	231	0	*
18	2	0	0	0	20	0	2	0	*
19	3×10^6	5×10^7	7×10^7	3×10^6	*	*	*	*	*
20	1	0	0	0	1	0	1	0	9
21	2×10^6	2×10^6	1×10^6	1×10^5	9×10^6	2×10^6	1×10^6	8×10^5	1×10^7
22	0	0	0	0	*	*	*	*	*

estimates of low-risk doses using the SW model are highly sensitive to estimates of the threshold parameter α.

4. Discussion

A general procedure, which may be able to accommodate biological theory, has been developed for deriving microbial dose-response models. Using this procedure, a number of dose-response models have been developed from rudimentary principles of biology and statistics, and they have been shown to provide acceptable descriptions of dose-response relationships observed for a variety of food-borne pathogenic micro-organisms. However, it is apparent from the foregoing exercise that it would be most difficult to settle on a single dose-response model as the best universal model to describe all types of microbial dose-response data. Also, for a given set of data, it would be difficult to choose from among several models that adequately describe the data in order to make predictions of risk at doses below the data range. Hence, the issue of model uncertainty cannot be resolved simply by the development of additional dose-response models. However, the availability of a number of plausible models that have the ability to describe observed data on micro-organisms permits the calculation of a range of risk estimates to help quantify the degree of model uncertainty present in a risk assessment.

One of the ultimate goals of this research has been to provide a formal statistical approach for directly and objectively incorporating model uncertainty into risk assessment (Kang *et al*., 2000). To do this, one does need a variety of plausible models. Existing models such as the BP, LN, LL and WG models, along with the new models introduced here, such as the WE, EG and SW models, provide a good starting set for developing a formal strategy. The primary way that model uncertainty is envisioned to be addressed is in the calculation of upper confidence limits on risk for fixed doses of micro-organisms and lower confidence limits on doses for fixed levels of risk (so-called benchmark doses). Seldom are central estimates of risk (dose) from dose-response models used to set regulatory levels, whether for chemicals or microbes. Rather, statistical confidence limits are employed in order to account for experimental variation. Hence, it is anticipated that incorporation of model uncertainty will be done in conjunction with the incorporation of uncertainty due to experimental variation. The fact that vastly different doses were estimated by different models to correspond to a risk of 0.01 for certain data sets in Table 3 illustrates the need to account for model uncertainty in microbial risk assessment. The method proposed by Kang *et al*. (2000) for incorporating model uncertainty into microbial risk assessment is to use a weighted average of estimated doses (risks) produced by various models. For some data sets, there will not be enough dose levels to include three-parameter models in the estimation process. Even if sufficient doses are available, it may not be necessary, or even desirable, to include three-parameter models, provided the suite of two-parameter models is sufficiently diverse. In particular, inclusion of three-parameter models that represent generalizations of two-parameter models in the suite is not recommended.

A feature of the SW model not present in the other models studied is a dose threshold. The issue of dose thresholds is currently being debated in microbial risk assessment, just as it has been for quite some time in chemical risk assessment. It seems important that a formal strategy for incorporating model uncertainty should consider threshold models. Of course, a threshold can be incorporated into existing two-parameter nonthreshold models, just as was done by Marks *et al*. (1998) in the BP model. However, as was seen for some of the data sets examined here, reliable estimation of a threshold parameter can be a difficult statistical problem. It should be clarified that the concept of a threshold need not refer to the individual infectivity probability. Rather, it can mean a population threshold in the following sense. It may be that a single micro-organism can infect an individual if that micro-organism survives the stomach and is able to penetrate the small intestine. However, it may require that a substantial number of micro-organisms be introduced into the stomach before there is a non-zero probability that one or more will survive to enter the small intestine. It is another goal of this ongoing research to develop biologically plausible dose-response models based on concepts of survival in the stomach, growth in the intestine and infection in the blood. Such models could help to alleviate some of the model uncertainty in the risk assessment process.

One distinguishing feature of all the models discussed here, except the EE model, is their prediction of zero probability at zero dose. This feature is different from all models used in chemical risk assessment. Presumably, subjects are free from any chance of infection except for the introduction of a specific pathogenic micro-organism. Although the EE model allows for background risk, it was seen to be one of the poorest fitting models for the data sets examined. The issue of non-zero background might become more important as the practice of microbial risk assessment develops.

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References

Armitage, P., Meynell, G.G., and Williams, T. (1965) Birth-death and other models for microbial infection. *Nature*, **207**, 570–72.

Buchanan, R.L. and Whiting, R.C. (1996) Risk assessment and predictive microbiology. *Journal of Food Protection, Supplement*, 31–6.

Farber, J.M., Ross, W.H., and Harwig, J. (1996) Health risk assessment of *Listeria monocytogeneses* in Canada. *International Journal of Food Microbiology*, **30**, 145–54.

FDA, USDA, EPA, and CDC (U.S. Food and Drug Administration, U.S. Department of Agriculture, U.S. Environmental Protection Agency and Centers for Disease Control and Prevention) (1997) *Food Safety from Farm to Table: A National Food Safety Initiative*. Report to the President.

Furumoto, W.A. and Mickey, R. (1967) A mathematical model for the infectivity-dilution curve of tobacco mosaic virus: theoretical considerations. *Virology*, **32**, 216–23.

Haas, C.N. (1983) Estimation of risk due to low doses of microorganisms: a comparison of alternative methodologies. *American Journal of Epidemiology*, **118**, 573–82.

Haas, C.N., Rose, J.B., and Gerba, C.P. (1999) *Quantitative Microbial Risk Assessment*, Wiley, New York, p. 449.

Holcomb, D.L., Smith, M.A., Ware, G.O., Hung, Y.-C., Brackett, R.E., and Doyle, M.P. (1999) Comparison of six dose-response models for use with food-borne pathogens. *Risk Analysis*, **19**, 1091–100.

Kang, S.-H., Kodell, R.L., and Chen, J.J. (2000) Incorporating model uncertainties along with data uncertainties in microbial risk assessment. *Regulatory Toxicology and Pharmacology*, **32**, 68–72.

Marks, H.M., Coleman, M.E., Lin, C.J., and Roberts, T. (1998) Topics in microbial risk assessment: dynamic flow tree process. *Risk Analysis*, **18**, 309–28.

Pinsky, P.F. (2000) Assessment of risk from long term exposure to waterborne pathogens. *Environmental and Ecological Statistics*, **7**, 155–75.

Teunis, P.F.M., van der Heijden, O.G., van der Giessen, J.W.B., and Havelaar, A.H. (1996) The dose-response relation in human volunteers for gastro-intestinal pathogens. Report Number 284550002, National Institute of Public Health and the Environment (RIVM), Bilthoven, The Netherlands.

Teunis, P.F.M., Nagelkerke, N.J.D., and Haas, C.N. (1999) Dose response models for infectious gastroenteritis. *Risk Analysis*, **19**, 1251–60.

Worcester, J. (1954) How many organisms? *Biometrics*, **10**, 227–34.

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