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Application of Elements of Microbiological Risk Assessment in the Food Industry Via a Tiered Approach

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ABSTRACT

Food safety control is a matter for concern for all parts of the food supply chain, including governments that develop food safety policy, food industries that must control potential hazards, and consumers who need to keep to the intended use of the food. In the future, food safety policy may be set using the framework of risk analysis, part of which is the development of (inter)national microbiological risk assessment (MRA) studies. MRA studies increase our understanding of the impact of risk management interventions and of the relationships among subsequent parts of food supply chains with regard to the safety of the food when it reaches the consumer. Application of aspects of MRA in the development of new food concepts has potential benefits for the food industry. A tiered approach to applying MRA can best realize these benefits. The tiered MRA approach involves calculation of microbial fate for a product and process design on the basis of experimental data (e.g., monitoring data on prevalence) and predictive microbiological models. Calculations on new product formulations and novel processing technologies provide improved understanding of microbial fate beyond currently known boundaries, which enables identification of new opportunities in process design. The outcome of the tiered approach focuses on developing benchmarks of potential consumer exposure to hazards associated with new products by comparison with exposure associated with products that are already on the market and have a safe history of use. The tiered prototype is a tool to be used by experienced microbiologists as a basis for advice to product developers and can help to make safety assurance for new food concepts transparent to food inspection services.

Food safety control and management approaches address the total food supply chain, and information on whether foods are safe can be obtained from various sources. One such source is microbiological risk assessment (MRA) studies that have been conducted over the last few years for a number of relevant pathogen-commodity combinations by national and international bodies such as the Food and Agriculture Organization, World Health Organization, U.S. Food and Drug Administration, and European Union *(3, 9, 10).* These MRA studies have generated risk estimates for pathogen-commodity combinations using data from one or more countries. This information can be used by governments to decide whether current practices provide safe foods or whether food safety improvements are needed. The findings of MRA studies should be presented in a structured and transparent way so that governmental risk managers and other stakeholders can discern what factors determine the risk estimate. Such factors can include the use of certain raw materials or ingredients, processing technologies applied, or consumer preparation. Thus, MRA studies provide risk managers with detailed information that should be helpful for determining which measures would be most relevant if the risk were judged unacceptable. The setting of national food safety policy in the framework of risk analysis is a developing concept. This approach has been promoted by Codex Alimentarius and has gained increasing international response *(5).* The three pillars of risk analysis are risk assessment, risk communication, and risk management *(4).* Through the adoption of the framework, governmental risk managers are provided in principle with a more open view of national food safety policy *(6).*

MRA studies are very generic because they are developed for a range of food industries and food chains that market a certain type of product. They generally involve typical or representative food chains, processing technologies, and contamination data. However, individual food industries (e.g., producers, manufacturers, processors, handlers, vendors, and caterers) must manage the safety of their products at a much more specific level, using suitable food safety management systems. The hazard analysis critical control point (HACCP) system is a food safety management system that is applied by many food industries to systematically control hazards in the production and marketing phase. HACCP systems consider very specific areas of food production, such as the structure of the food chain supporting a specific operation, the level of contamination of raw materials used, processing technologies deployed, shelf life, and expected consumer use. This level of specificity is key to achieving an appropriate food product and process design and to successful marketing of a safe product.

Aspects of MRA can be used in the specific situation of a food industry. Here, we present an application for the establishment of safe product and process designs suitable for new product concepts or new consumer markets. This

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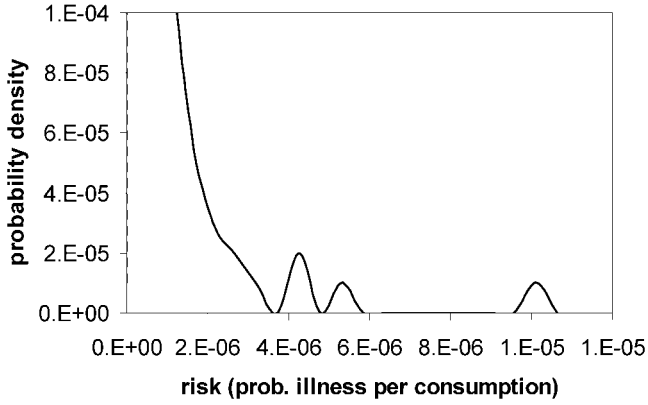

FIGURE 1. *Outcome of a hypothetical risk assessment study of* Listeria monocytogenes *in a frozen neutral product (pH 7).*

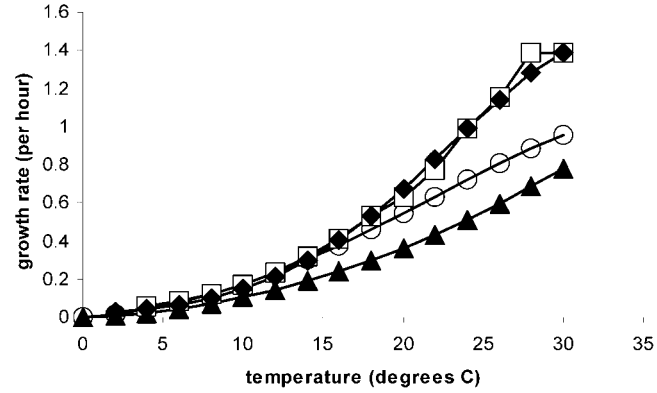

FIGURE 2. *Growth rates for* Listeria monocytogenes *predicted by various models at various temperatures, pH 7, and* a_w *0.99.* □*, Pathogen Modeling Program (U.S. Department of Agriculture, Eastern Regional Research Center, Wyndmoor, Pa.);* ♦*, Food MicroModel (Leatherhead Food Research Association, Leatherhead, Surrey, UK);* ○*, CTPM (20) using worst-case parameters;* ▲*, gamma model (28) using worst-case parameters. The worst-case parameters were as follows:* $T_{min} = -1.5$℃ *(11);* $T_{opt} = 35$℃ *(average value);* $T_{max} = 45$℃*;* $pH_{min} = 4.39$*;* $pH_{opt} = 7$*;* $pH_{max} = 9.4$*;* $a_{w_{min}} = 0.92$ *(12).*

application provides another tool for food safety professionals in industry but does not in any way replace existing food safety management systems.

APPLICATION OF ASPECTS OF MRA IN THE FOOD INDUSTRY

Elements of MRA can be used as input to HACCP plans where they help industry to assess realistic hazards, possible consumer exposure levels for particular product formulations or processing technologies, and suitable measures for hazard control. In a quantitative stochastic MRA, uncertainty and variability are represented in the outcome of the risk estimate (Fig. 1) and can cause difficulties in data interpretation. For instance, it may be difficult to interpret a probability (*y*-axis) of a probability (*x*-axis). However, uncertainty and variability must be accounted for when making decisions on the safety of a product concept and the suitability of hazard control measures. The decision-making process can be facilitated by the approach suggested by Van Gerwen et al. *(23).* With this approach, the decision maker sets (i) an acceptability criterion (AC) for a risk and (ii) an acceptable percentage (AP) regarding the area of the risk distribution that falls below the AC. A possible AC is that the acceptable probability of illness per consumption is 10^{-8}. The accompanying AP could then state that at least 95% of the total area of the curve must be below the risk estimate of 10^{-8}. Thus, when a probability of illness per consumption is $>10^{-8}$ in $<5\%$ of the cases, the risk is considered acceptable. In the example given in Figure 1, the criteria for acceptance are met because (i) the mean of the distribution, 2.35×10^{-9}, is lower than the acceptability criterion ($AC = 10^{-8}$) and (ii) the distribution area that is below 10^{-8} is greater than the acceptable percentage ($AP = 95\%$) because 99.9% of the area is below 10^{-8}.

Instead of the distribution area, a weighted area can be considered for the AP. This approach is appropriate when small percentages associated with high probabilities of illness determine the risk, e.g., the small probability in the right tail of the distribution (*x*-axis) pictured in Figure 1. Weighting (W) is linked to the severity of the hazard and AC. In this example, $W = \exp(10^5 \cdot \text{risk})$. Again, 99.9% of the weighted area is below the AC, so the product can be accepted according to the defined criteria. These two parameters facilitate decisions on the acceptability of the risk outcomes regardless of the shape of the risk distribution.

Important tools in MRA are predictive mathematical models of microbial inactivation, survival, and growth. The literature on predictive modeling is extensive, and benefits and pitfalls have been well described *(18, 22).* Because no model is able to accurately predict microbial responses under all circumstances, van Gerwen et al. *(25)* suggested comparing the results of various models. The relevance of comparing predictions obtained with different models is shown in Figure 2. In this example, model predictions for the growth rate of *Listeria monocytogenes* in a neutral product (pH 7, water activity [a_w] 0.99) show considerable differences at particular temperatures. Thus, predictive models can be used reliably for robust product and process design only when they have been validated in the specific product concerned. Examples of validation studies that compare different models have been published *(1, 15, 21, 28).* The existing models still cover only a few existing products, product formulations, and processes, and only relatively few of the many potential hazards in foods have been modeled extensively. Water droplet size (relevant to foods containing water in oil emulsions) and most of the widely used preservative factors (e.g., acetic, lactic, and sorbic acids) have been modeled and validated in real products for relevant pathogens and spoilage organisms to only a limited extend *(2, 13, 16),* which restricts the active use of such predictive models by industry. Nevertheless, in principle, these models support quantification of hazard levels in the food production chain.

The tiered approach proposed here includes aspects of MRA in an attempt to extend the benefits of predictive models to other areas relevant to the food industry. It allows (i) a structured analysis of food safety, (ii) quantification of microbial fate throughout the production chain, (iii) quan-

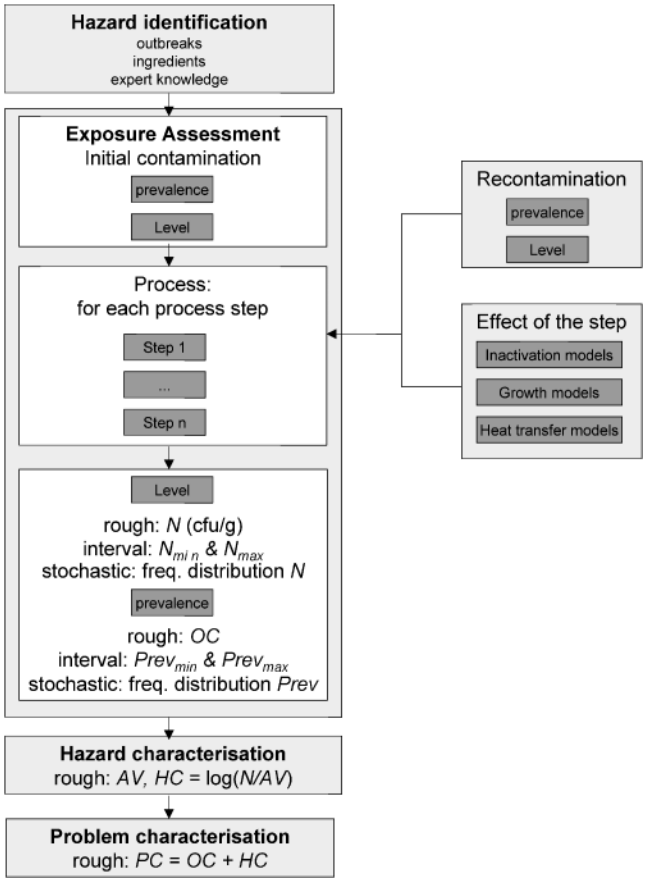

FIGURE 3. *Elements of the MIRACLE approach for microbiological risk assessment. OC is a logarithmic measure for the probability of survival; HC is a log-linear description of the dose-response relation, using the rough exposure estimate* N *and AV (CFU/g); AV is a measure of pathogen infectivity;* PC *is a logarithmic measure for the risk. These characteristics have been extensively described by van Gerwen et al. (25).*

tification of the relevance of hazards, (iv) identification of the major risk-determining phenomena, and (v) evaluation of relevant process scenarios in relation to (end)product specifications.

The approach was not developed to give precise answers to particular risk questions; instead, it supports ranking of risks associated with different products and identification of the factors critical for determining the risk. The approach is referred to as MIRACLE: microbiological risk assessment as a tool to understand the cliff edge. In this regard, *cliff edge* refers to the boundary between a product being microbiologically stable (meaning safe and not spoiled when consumed) and it being unstable. The MIRACLE approach was developed by examining several hypothetical foods (frozen foods, ice cream) that were relatively simple with regard to microbiological issues. MIRACLE has been implemented as a prototype system in an Excel 97 spreadsheet (Microsoft Corporation, Redmond, Wash.) that uses @Risk (version 3.5e, Palisade Corporation, Newfield, N.Y.) as an add-in.

MIRACLE

The elements of the MIRACLE system are shown in Figure 3. The approach starts with hazard identification. Hazards can be identified by three information sources: (i) reported outbreaks related to the product and/or product ingredients, (ii) reported presence of pathogens on food commodities and product ingredients, and (iii) expert experience with relevant pathogens. Data from the first two sources have been collected from the literature and entered in databases *(24).*

The first tier of MIRACLE is a rough semiquantitative risk assessment *(25).* The assessment is conducted using available data and knowledge regarding the specific conditions of the food production chain or the food manufacturing process. Where data and knowledge are not available, for instance with new product concepts, assumptions are made based on available scientific and technical information. This tier provides rough estimates of risks related to the consumption of a food product and identifies risk-determining factors. If conditions or assumptions in the first tier affect the outcome to a large extent, they must be evaluated in a more detailed study. To assess the impact of events and assumptions, use is made of (i) scenario studies, such as worst /average/ best-case scenarios *(30),* and (ii) knowledge rules *(25).* If a sufficient margin of safety is evident, further elaboration of the exposure assessment may not be required *(8).*

The second tier focuses on the conditions or assumptions that strongly affect the outcome of the assessment. The second tier only includes exposure assessment since exposure assessment is sufficient in the industrial context. Tools used are interval analysis and stochastic analysis. Interval analysis involves calculation of the minimum and maximum values for the concentration and prevalence of hazards in food products. Theoretically, the absolute minimum and maximum values should be calculated, but for practical applicability it is adequate to use realistic ranges. Interval analysis can be used to clearly identify parameters that are not risk determining, even for parameters that have a wide range of possible values. This simple technique allows calculations using default values when relevant data are not available. Table 1 summarizes default inputs of the interval and stochastic analyses that can be used when data or specific models are not available. The impact of these interval analysis default values on the exposure estimate is easily identified (Fig. 4). Stochastic analysis involves input parameters that are described as frequency distributions. Monte Carlo simulation is a frequently used technique in stochastic analysis *(26).* When no data are available, the MIRACLE system does not perform stochastic analysis because the use of frequency distributions requires additional assumptions, i.e., on the probabilities of (default) values. The uniform distribution assumes that all values between the absolute minimum and maximum values have an equal probability, whereas the normal distribution assumes symmetric probabilities around the mean. Checking the validity of an assumed probability distribution may be difficult in practice particularly when no validated experimental data are available. As an example, parameters used to estimate growth rate by the combination of the cardinal temperature and pH model (CTPM) and the gamma model *(20, 29)* can be derived from the literature. These input parameters, e.g.,

TABLE 1. *Default values and procedures for interval and stochastic analyses in the absence of data or specific models*

Step	Default interval input	Default stochastic input
Level of contamination	$1–10^4$ CFU/g	Use worst-case point estimates
Prevalence of contamination	10^{-20}–1	Use worst-case point estimates
Level of recontamination	$1–10^4$ CFU/g	Use worst-case point estimates
Prevalence of recontamination	10^{-20}–1	Use worst-case point estimates
Process steps	Minimum and maximum values for process parameters set per process step	Use target values for process parameters as point estimates
Growth	Use combined CTPM-gamma model *(20, 29)*, preferably with interval ranges for parameters; growth curve by exponential growth model	Use combined CTPM-gamma model *(20, 29)* for point estimate; growth parameters equal to parameters in rough assessment *(25)*; growth curve by exponential growth model
Inactivation	Same inactivation parameters as in rough assessment *(25)*; inactivation curve by exponential model	Same inactivation parameters as in rough assessment *(25)*; inactivation rate calculated with normally distributed log(*D*); inactivation curve by exponential model

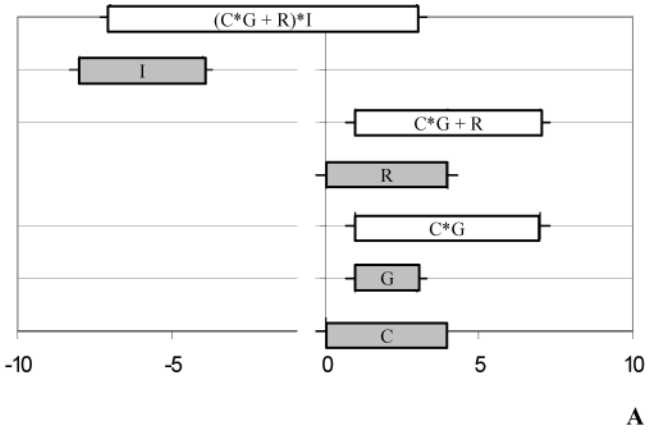

FIGURE 4. *Input parameters (shaded) and outcomes (open) of interval analysis. The* x*-axis represents log values of the factors: C is level of contamination; G is a growth factor (e.g., 10-fold increase); R is level of recontamination; I is an inactivation factor (e.g., change of concentration with factor 10^{-4}). (A) Interval analysis with default levels of (re)contamination (see Table 1). (B) Specific levels of (re)contamination.*

T_{min} is the minimum growth temperature, are generally provided as point estimates without confidence intervals *(12)*. The estimated growth rate is therefore also a point estimate. There are no clear grounds for assuming a frequency distribution for the growth rate. Therefore, in cases where no validated experimental data are available, the practical relevance of probability estimates may be low. Quantification of probabilities may even be misleading by providing apparent security concerning the occurrence of extreme values. Figure 5 shows results of stochastic analysis for a hypothetical example. The range of input values was equal for Figures 4 and 5. As expected, the range of outcomes of the interval analysis is wider than that of the stochastic analysis.

In the following sections, we describe the principles used in interval and stochastic analyses concerning the various aspects of exposure assessment that are used in the current software version of the system.

Level of contamination. As default, it is assumed that all ingredients enter the process simultaneously at the start.

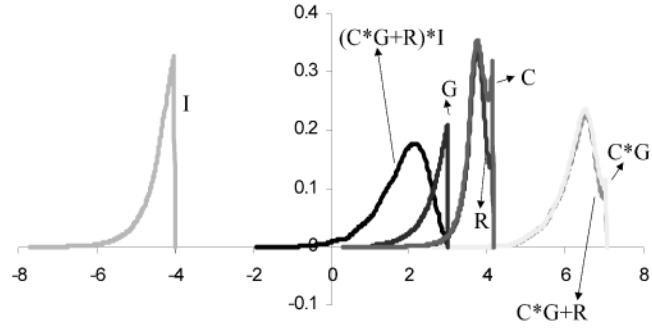

FIGURE 5. *Results of stochastic analysis. C is the level of contamination; G is a growth factor; R is level of recontamination; I is an inactivation factor. The input distributions were normal(5,000, 2,500) for C and R, uniform(10, 1,000) for G, and uniform(10^{-8}, 10^{-4}) for I. The outcomes were log transformed for plotting. The values of the normal distributions for (re)contamination were chosen to approximate an interval of 1 to 10,000.*

If a contaminated ingredient enters the process at a later stage, the data can be entered as recontamination at that stage. MIRACLE uses raw material monitoring data from the food business for quantitative description of microbial contamination. The level of contamination attributed to an ingredient is weighted by multiplying the level of contamination (expressed as CFU/g) by the fraction of the ingredient in the final product.

For the interval analysis, two principles apply. The first is that the minimum level is the lowest weighted level for all ingredients. However, if the prevalence of contamination for a particular ingredient *m* is equal to 1 (contamination occurs in this ingredient in 100% of the cases), then the weighted level of ingredient *m* is taken as the minimum level of contamination in the product. If more ingredients have a prevalence of 1, then the sum of the weighted levels of these ingredients is taken as the minimum level. The maximum level is the sum of weighted levels of all ingredients. The second principle is that the minimum level is set at 0 CFU/g and the maximum at 5 CFU/g in case contamination is below the level of detection. This principle follows from standard statistical tables for the Poisson distribution: when zero cells per gram have been measured (Poisson parameter $\lambda = 0$), there is a reasonable (0.01) probability that the actual level was 5 CFU/g.

For the stochastic analysis, three principles are adhered to. First, a Poisson distribution is assumed when contamination levels are very low, which is generally true for pathogenic organisms. Second, it is assumed that all samples (1 ml) come from the same Poisson distribution, with parameter λ_{sample}. This principle allows pooling of the samples. It is assumed that λ_{sample} is the sum of colonies measured in all samples, which represents a worst-case assumption. The upper confidence limit of λ_{sample}, designated λ_{upper}, is derived from a standard statistical table. The λ_{upper} value is used as input for further calculations: λ_{upper} is divided by the total number of samples to calculate the Poisson parameter per milliliter, λ_{ml}, and λ_{ml} is recalculated to a Poisson parameter for the ingredient by multiplying it by the product quantity, and the fraction of the ingredient in the product. Third, the Poisson distributions for the various ingredients are summed to result in a distribution that describes the initial contamination level. This third principle applies in cases of high levels of contamination where the Poisson distribution converges to a normal distribution *(26).*

Prevalence of contamination. Prevalence is defined as the fraction of samples positive for a particular contaminant, regardless of its concentration. Prevalence in batches and products is assumed to be equal to prevalence in the samples taken from them. The prevalence of initial contamination (Pic) is the likelihood of contamination of one or more of the ingredients. Pic is calculated as follows: $\text{Pic} = 1 - (1 - \text{Pic}_{i1})\cdot(1 - \text{Pic}_{i2})\cdot(1 - \text{Pic}_{i3}) \ldots$, etc., where Pic_{i1} is the prevalence for ingredient 1, Pic_{i2} is the prevalence for ingredient 2, etc.

For interval analysis, two principles apply. First, if there is one data set per ingredient, there is one estimate of prevalence, meaning that the minimum prevalence is equal to the maximum prevalence. Second, if all data are below the detection level and thus no positive samples have been found, the default range for prevalence (Table 1) is assumed.

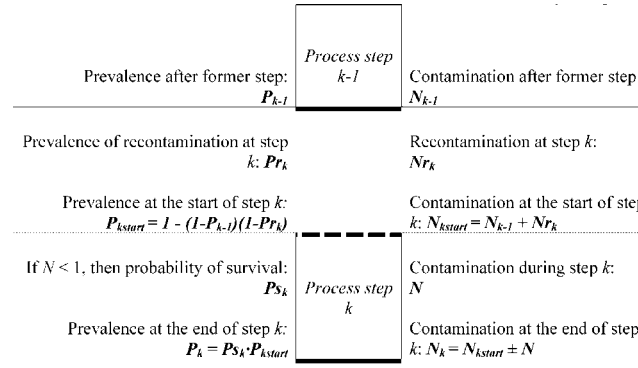

FIGURE 6. *Schematic representation of parameters used by the MIRACLE approach in exposure calculations with regard to recontamination.*

For stochastic analysis, it is assumed that the number of samples is *n* and the number of positive samples is *s.* The frequency density of prevalence can be described by a beta distribution (see equation 1) *(26)* with domain $0 \leq x \leq 1$.

$$f(x) = \frac{x^{\alpha-1}(1-x)^{\beta-1}}{\int_0^1 t^{\alpha-1}(1-t)^{\beta-1}\,dt} \qquad (1)$$

The parameters of the Beta distribution are approximated as $\alpha = s + 1$ and $\beta = n - s + 1$ *(26).*

Recontamination. Symbols used to estimate recontamination at step *k* are pictured in Figure 6. The following assumptions are made regarding recontamination.

1. It is assumed that recontamination takes place at the start of the relevant process step.
2. Prevalence at the start of step *k*, called P_{kstart} (see Fig. 6), is described by the equation $P_{\text{kstart}} = (1 - P_{k-1})\cdot(1 - \text{Pr1}_k)$. This equation expresses the probability of bacterial presence either because of recontamination at the step examined or because of lingering contamination from a former process step. If recontamination comes from various sources at one step, the equation is extended as $P_{\text{kstart}} = (1 - P_{k-1})\cdot(1 - \text{Pr1}_k)\cdot(1 - \text{Pr2}_k) \ldots$, etc.
3. If inactivation during the process step leads to a decrease in contamination, so that $N < 1$ (with *N* expressed in CFU/g), then a probability of survival is introduced. For instance, when $N = 0.01$ CFU/g, the probability for survival in step *k*, termed Ps_k, is 0.01 and $N_k = 1$ CFU/g *(25).*

Often, it is difficult to assess the level and prevalence of recontamination because the source of recontamination generally is unknown. The following procedures can be used to theoretically evaluate consequences of recontamination at various stages in the food supply chain. They can

also be used to calculate the consequences of a sporadic source of recontamination.

Level of recontamination. For interval analysis, it is assumed that $N_{kstart} = N_{k-1} + Nr_k$ (Fig. 6), except when recontamination is a rare event but introduces a high concentration of the contaminant. For $10N_{k-1} \leq Nr_k$ and $P_{k-1} \geq 10Pr_k$, recontamination is ignored and $N_{kstart} = N_{k-1}$ whereas $P_{kstart} = P_{k-1}$. Separate calculations should be performed for Nr_k and Pr_k to understand the consequences of rare recontamination.

For stochastic analysis of recontamination, the same principles apply as for stochastic analysis of the level of contamination. When recontamination is rare but at a high level, techniques described for risk assessment of extreme events may be useful for the selection of an appropriate frequency distribution *(14, 17).*

Prevalence of recontamination. For interval analysis, MIRACLE uses calculations other than the equation for P_{kstart} (Fig. 6) in the following cases.

1. If $N_{k-1} \geq 10Nr_k$ and $10P_{k-1} \leq Pr_k$, then N is estimated using the level of recontamination (Nr_k) and the prevalence of recontamination ($P_{kstart} = Pr_k$). This process gives the most likely scenario because the probability of recontamination is much larger than the probability of contamination from the former step. Again, separate calculations should be performed for N_{k-1} and P_{k-1} to evaluate the impact of rare contamination.
2. If $10N_{k-1} \leq Nr_k$ or $10N_{k-1} \geq Nr_k$, and $P_{k-1} \approx Pr_k$, then $P_{kstart} = Pr_k$ and $P_{kstart} = P_{k-1}$, respectively.
3. If $10N_{k-1} \leq Nr_k$ and $Ps_{k-1} \geq 10Pr_k$, then N is estimated using N_{k-1} and $P_{kstart} = P_{k-1}$ to obtain the most likely scenario. To understand the consequences of rare recontamination, separate calculations should be performed for Nr_k and Pr_k.

The stochastic analysis is similar to stochastic analysis for the prevalence of contamination.

Process steps. Processing technologies based on heat are frequently used in food industry for product manufacture, and temperature is one of the most relevant determinants of the potential of microbes to grow or to become inactivated. Heat transfer is estimated as suggested by van Gerwen et al. *(25).* For simplicity of calculations, one-way heating and cooling via the shortest distance to the center of the product (characteristic length, L) is assumed. Other important assumptions are that the product is homogeneous and that the heat resistance of the package of the product is negligible. For temperature gradients, microbial growth is estimated over increasing time intervals: intervals of 10 s during the first minute of a processing step, 30 s in the second minute, and 60 s for longer times. It is advisable to check the influence of the time intervals chosen by calculating the outcome using other time intervals. Under conditions of conduction cooling, when the temperature drops below the inactivation range and reaches the growth range, it is assumed that no growth occurs. The rationale for this assumption is that the time during which the temperature per process step is in the growth range is probably too short for the organism to go beyond the lag phase.

For interval analysis, six principles are applied. First, minimum and maximum values per process step are set for temperature, time, pH, and a_w provided these are constant during the process step. Second, when conductive heating is applied until the optimal growth temperature (T_{opt}) is reached, minimum growth is estimated at the coldest spot, which is the center of the product. In contrast, maximum growth is estimated at the surface of the product, which is the first area to reach T_{opt}. When heating results in temperatures higher than T_{opt} but still lower than the maximum growth temperature (T_{max}), minimum growth is estimated at the surface because the temperature there is the first to deviate from T_{opt}. Third, if conductive cooling results in $T < T_{opt}$, minimum growth is estimated at the surface of the product. When cooling brings temperatures below T_{max} but still above T_{opt}, minimum growth is estimated in the center because the temperatures there deviate for the longest time from T_{opt}. Fourth, when conductive heating results in inactivation temperatures, i.e., $T > T_{max}$, the minimum contamination level is estimated at the surface of the product because temperatures there rise the fastest, resulting in highest inactivation. The maximum value for process time is used because this gives maximum reduction. The maximum contamination level is estimated at the center of the product using the minimum process time value. Fifth, for conductive cooling and $T > T_{max}$, the minimum contamination level is estimated at the center of the product using the maximum time value, whereas the maximum level is estimated at the surface of the product using the minimum time value. Sixth, when convective heating or cooling is applied, temperature differences in the product are assumed to be negligible. Consequently, there is no difference between minimum and maximum growth or inactivation by temperature.

For stochastic analysis, the following two principles are adhered to. First, random fluctuations in temperature, time, pH, and a_w that do not build up gradients can generally be described by a normal distribution. When data on random fluctuations in temperature, time, pH, and a_w are not available, the minimum and maximum process target values are used as point estimates. Second, if gradients exist, the time for the temperature gradient is set at the average time of the process step (point estimate). This approach is deterministic, and stochastic analysis would sample from the time distribution. Temperature gradients are estimated at six points in the product: center, center + $\frac{1}{5}L$, center + $\frac{2}{5}L$, center + $\frac{3}{5}L$, center + $\frac{4}{5}L$, and surface $- 0.01L$. For simplicity, it is assumed that the contaminating bacteria are homogeneously distributed in the product. For a step with a temperature gradient, the total number of organisms present at the start (N_{kstart}) is divided by 6. For each point in the product, the change in number is then estimated. For point 1, $N_1 = change_1 \cdot N_{kstart,1}$; for point 2, $N_2 = change_2 \cdot N_{kstart,2}$, . . . ; for point 6, $N_6 = change_6 \cdot N_{kstart,6}$. The six resulting estimates are summed to give N_k, so $N_k = sum(N_1, \ldots, N_6)$. It is advisable to check the influence of using exactly six points in the product by

recalculating the outcome using a different number of points. The results should be compared until there is sufficient confidence in the reliability of the results, i.e., when results deviate by <5%.

Microbial modeling. Predictions resulting from mathematical models that have not been validated in an appropriate product (the actual product under study or one that is sufficiently similar in terms of microbiology) should be interpreted with care. Before deciding on the use of a particular model, the researcher should evaluate whether using different types of models significantly affects the outcome of an exposure assessment. Uncertainty and variability related to microbial growth and inactivation are generally not included in most growth models *(18).* Comparison of models gives a rough qualitative impression of the uncertainty in model predictions. For instance, when the predictions are far apart (Fig. 2), it is uncertain which model predicts reasonably well.

Modeling growth of microbial contaminants. When no validated models or experimental data are available, the growth rate is best estimated with the CTPM *(20)* for temperature and pH effects and the gamma model *(29)* for a_w effects.

For interval analysis, two key principles apply. First, when model predictions for growth rate are available, expert judgement is used to establish sensible interval ranges. When the model predictions do not seem realistic to the expert but relevant experimental data are available, then the minimum and maximum growth data under relevant circumstances are used in the calculation. Second, when using the CTPM in combination with the gamma model, intervals for growth parameters should be collected from the literature. These values also should be critically reviewed by an expert. As an example, although the minimum growth pH (pH_{min}) reported for *Staphylococcus aureus* is 2.6 *(27),* a microbiological expert would know from experience and published data *(12)* that *S. aureus* will not grow below pH 4.2 to 4.5 in food products containing, for instance, organic acids. Thus, for foods containing relevant amounts of organic acids, it does not make sense to use a pH value of 2.6 for assessing the growth rate. The predicted values for maximum growth rate are the same as in the first tier of MIRACLE (worst case).

For stochastic analysis, experimental data are required when validated model predictions are not available. A probability distribution can be fitted to the measured growth data, and the best fit can be chosen. Distributions other than the best fit can be used when there are clear indications for doing so. When the number of data points is too low to reasonably fit a frequency distribution, the mean and variance of the data can be estimated and used as input parameters to the normal distribution. When both growth data and specific models are not available, worst-case point estimates for the growth parameters are used in the CTPM and gamma models. Preferably, the approach of Delignette-Muller and Rosso *(7)* is followed where empirical distributions are fitted to reported growth parameters when more data are available from one resource. However, generally only point estimates are available from the literature *(12).*

Modeling inactivation of microbial contaminants. When no validated models or experimental data are available, the inactivation rate is estimated with *D* values (decimal reduction times) from the literature.

For the interval analysis, the maximum interval scenario uses the worst-case inactivation rate as used in the first tier (rough assessment). The worst-case inactivation rate results in minimum inactivation, i.e., maximum contamination levels of remaining microorganisms. The minimum interval scenario uses the average inactivation rate as used in the rough assessment. The best-case inactivation rate was not used because an underestimation of exposure would probably result. When specific models and/or experimental data are available, the situation is comparable as described in the section above on modeling growth.

For stochastic analysis, when neither validated model predictions nor specific experimental data are available, the average log(*D*) is estimated as described by Van Gerwen et al. *(25).* It is assumed that log(*D*) is normally distributed. The parameters of the distribution follow from the linear regression: $\mu = \log(D)$; $\sigma^2 = \text{var}[\log(D)]$. The inactivation rate *k* is calculated from *D (25).*

Survival. Survival modeling has not been implemented in the current version of the MIRACLE tool. The procedure for prediction of survival will be similar to those for growth and inactivation.

Output of MIRACLE. The system estimates the probable concentration of a particular contaminant in the final food product, which is comparable to an exposure assessment in an MRA study. The outcomes of interval and stochastic analyses can be compared to determine whether the estimated exposure level is acceptable. For products already on the market with a safe history of use, data on contamination levels throughout the food production chain or the manufacturing process and data on the major risk-determining factors will be available. For new products or product concepts, such data will most often not be available and, therefore, assumptions are used that follow principles based on scientific and technical experience. The results of interval and stochastic exposure assessments will be similar whether actual experimental data for contamination and risk-determining factors are available or whether they have been simulated. Interval analysis will generally give a wider range of possible outcomes that may encourage targeted experimentation to establish the necessary data. Comparison of exposure assessments for existing products with those for new product concepts should allow food safety professionals a sufficient basis to decide whether the new product is acceptable in terms of food safety.

DISCUSSION

MRA studies increase the food industry's understanding of the rationale for control measures or other interventions proposed by governments to further increase food safety. These studies also can generate new insights rele-

vant to the food safety management systems that food industries use. Application of aspects of MRA may support food industry in the evaluation of the safety of new food products when experimental data and direct knowledge are lacking. A tiered approach to the application of elements of MRA was developed and tailored to the needs of the food industry. This approach is sufficiently pragmatic to allow decision making without extensive use of resources. It has been embedded in a spreadsheet that makes the procedure and the underlying principles user friendly. The procedure follows a generic process that allows comparison of product-pathogen combinations in different food groups or categories in terms of the potential exposure of consumers to particular hazards. The output of the tiered approach helps experts determine the appropriateness of a product and process design in terms of its microbiological safety. The system does not provide an absolute outcome, but it may reduce the effort needed to establish the conditions that assure that the product can be introduced on the market safely.

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