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International Journal of Food Microbiology 58 (2000) 213–221

INTERNATIONAL JOURNAL OF
Food Microbiology

www.elsevier.nl/locate/ijfoodmicro

Sensitivity analysis in quantitative microbial risk assessment

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Abstract

The occurrence of foodborne disease remains a widespread problem in both the developing and the developed world. A systematic and quantitative evaluation of food safety is important to control the risk of foodborne diseases. World-wide, many initiatives are being taken to develop quantitative risk analysis. However, the quantitative evaluation of food safety in all its aspects is very complex, especially since in many cases specific parameter values are not available. Often many variables have large statistical variability while the quantitative effect of various phenomena is unknown. Therefore, sensitivity analysis can be a useful tool to determine the main risk-determining phenomena, as well as the aspects that mainly determine the inaccuracy in the risk estimate. This paper presents three stages of sensitivity analysis. First, deterministic analysis selects the most relevant determinants for risk. Overlooking of exceptional, but relevant cases is prevented by a second, worst-case analysis. This analysis finds relevant process steps in worst-case situations, and shows the relevance of variations of factors for risk. The third, stochastic analysis, studies the effects of variations of factors for the variability of risk estimates. Care must be taken that the assumptions made as well as the results are clearly communicated. Stochastic risk estimates are, like deterministic ones, just as good (or bad) as the available data, and the stochastic analysis must not be used to mask lack of information. Sensitivity analysis is a valuable tool in quantitative risk assessment by determining critical aspects and effects of variations.

Keywords: Quantitative risk assessment; Sensitivity analysis; Food safety

1. Introduction

The microbial safety of food products depends on many factors such as the composition of the product, the process hygiene, and the storage and distribution conditions. A method that is becoming increasingly important for the control of food safety is quantitative risk assessment. This is a systematic, structured method to identify hazards and to estimate the risk. It is, however, impractical to quantitatively determine all aspects in great detail, in view of the wide range of factors involved. Furthermore, it should be realised that it is impossible to determine risks with high accuracy, due to many inaccurate and even unknown aspects.

Quantitative risk analysis will be the basis of

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0168-1605/00/$ – see front matter
PII: S0168-1605(00)00275-0

future food safety control systems, and will therefore become more and more important, particularly for international trade. It is a challenge to be able to make decisions on a more solid and transparent basis. This contrasts with present decisions, which are based on valuable expert knowledge, but also sometimes on non-validated anecdotal information. Supported by quantification, resources (research, sampling, hygiene, control) can better be targeted.

Modelling offers many possibilities in the quantitative estimation of spoilage and safety. The estimations obtained are estimations of the order of magnitude, based on qualitative and quantitative information. These estimations can give insight in important processes, help to determine the rate-determining steps, and make predictions during product development. Since rarely are all phenomena exactly quantifiable and known, it is important to determine the effect of changes in the assumptions. Sensitivity analysis will be described in three stages, as a valuable tool for determination of relevance of variations in factors.

2. Theory

Risk Analysis is a process to scientifically evaluate the probability of occurrence and severity of known or potentially adverse health effects resulting from human exposure to foodborne hazards (risk assessment); to weigh policy alternatives in the light of the results of the risk assessment and, if required, to select and implement appropriate control options (risk management); and to exchange information and opinions interactively among risk assessors, risk managers, and other interested parties (risk communication) (Lammerding, 1997). Risk assessment consists of (1) hazard identification, (2) exposure assessment, (3) hazard characterisation, and (4) risk characterisation. It is interesting to note that food scientists generally have as starting point the food product and reason forward towards the disease, whereas epidemiologists have as a starting point the disease and reason backwards to the food product (Fig. 1). Both views are of course valuable and should be used in parallel.

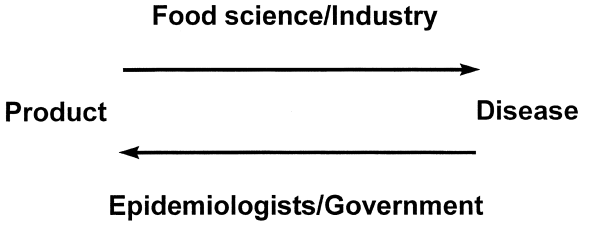

Fig. 1. Starting point of risk assessment.

3. Relevant aspects of the risk assessment steps

3.1. Hazard identification

Hazard identification is the identification of biological agents capable of causing adverse health effects that may be present in a particular food or group of foods (Lammerding, 1997). There are many pathogens and toxins able to represent a potential risk in foods, however disease only occurs in specific cases. Therefore, an educated selection has to be made of the relevant hazards on which to focus. This procedure will be mainly qualitative in nature and based on expert knowledge, databases and literature. There are however only limited data sources, which are also very diffuse. Apart from the risk of known potential hazards, also emerging or new unknown pathogens can form a potential risk.

For a food product the most obvious hazard(s) can be identified based on a combination of databases, reported cases, reported presence in the ingredients of the product, literature and expert knowledge (Van Gerwen et al., 1997).

3.2. Exposure assessment

Exposure assessment is the qualitative and/or quantitative evaluation of the likely intake of the biological agent via a food (Lammerding, 1997). The exposure depends on the occurrence in the raw materials, possible contamination, survival, re-contamination, and growth. For exposure assessment, both the level of the hazard in the food as well as the food intake are relevant. For food intake, consumption patterns must be known. The level of the hazard in the food can be estimated by using literature data, sampling, or by using predictive microbiology. Pre-

dictive models have been developed to describe the effect of factors in production and distribution on the growth or decline of microorganisms. Examples are Food Micromodel, Pathogen Modeling Program, or Ratkowsky type models (McMeekin et al., 1993). Models are simplified representations of reality taking into account main effects, and even for these main effects the models are not exact. Accordingly, there is more or less inaccuracy in the prediction. However, they are useful tools to quantify and to determine the order of magnitude and to get an insight into the kinetics of processes. Fig. 2 shows how in a chain of processes relevant stages are clearly detected.

The main problem in exposure assessment is often lack of sufficient, relevant, and accurate data. Detection of microorganisms might be affected by the sensitivity of the methods and is in many cases practically impossible (relevant numbers are below the detection limit, or frequency is so low that sampling is impossible). The estimation of the ingested numbers by predictive models also lacks sufficient data, since there are many unknown biological parameters (of the microorganism and of the food product) and unknown process factors (for example temperature distributions). Bacterial numbers in food can only be determined with a rather low accuracy (both sampling and prediction result in rather large inaccuracies, in the order of a factor 2 or 3, even sometimes 10), also, for food intake, order of magnitude estimations are generally sufficient. Almost all products are consumed at levels of about 100 g (range 30 and 300 g), products like spices excluded.

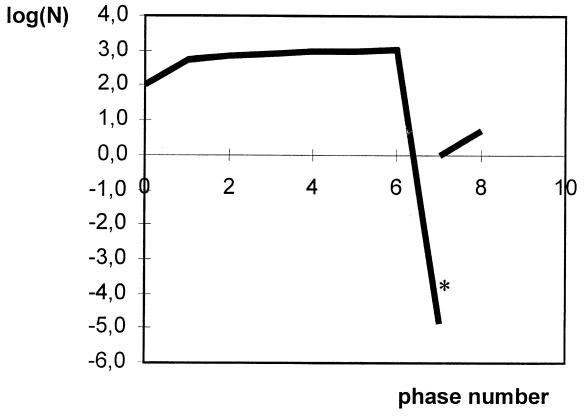

Fig. 2. Predicted development of numbers per product of *Salmonella* on chicken in various process stages. Cooking (phase number 7) is the most important stage. *: After heat treatment, the calculations were continued with 1 CFU product^{-1}, with a probability of survival of P_s.

3.3. Hazard characterisation

Hazard characterisation is the qualitative and/or quantitative evaluation of the nature of the adverse health effects associated with biological agents that may be present in food (Lammerding, 1997). The effect of an ingested dose can be determined by volunteer studies, animal studies, or by outbreak investigations. These methods to determine the relation between the ingested dose and the response have their advantages and disadvantages, but all yield useful information. Human volunteer studies often have been performed with high doses, for relatively low susceptible persons, but they show clearly in which range of dose, the risk of infection is likely. The results from animal studies have to be translated to humans, but can well be used to determine in some detail the shape of the curve, especially at low doses. Furthermore the orders of magnitude of changes in the curve due to for example age, feed status, and health can be determined. In outbreak investigations it is often difficult to determine the ingested doses and the exposed population, but they can yield real field information with relevant strains and situations. The best approach is to combine all these sources of information. One example of combination of epidemiological data and food survey data, is presented by Buchanan et al. (1997), where a dose response relation is derived by assuming that all cases of Listeriosis in Germany are caused by smoked fish. This then yields a worst-case estimate, but shows clearly that with a combination of different information sources a question can be answered. This answer again is not the definitive answer, but can be combined with other results to yield, in time, an even better dose response relationship. It should be noted that for dose response curves, the relevant range is generally far below $P=0.1$ (10% risk), therefore a log–log representation is generally the most relevant (Fig. 3).

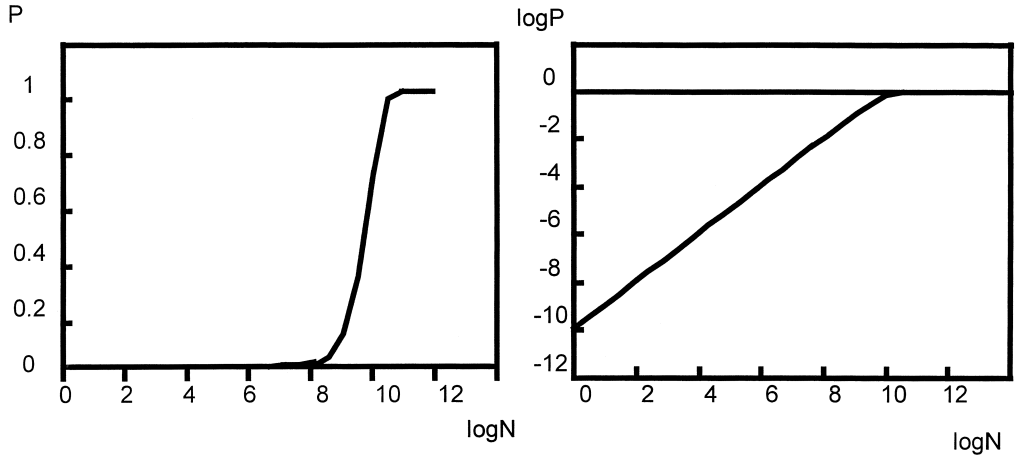

Fig. 3. Dose–response curve predicting the effect (probability of infection) of ingestion of a dose of *Listeria* for a susceptible population (Buchanan et al., 1997), on normal and log scale, showing that for low risks the log representation is the most relevant.

3.4. Risk characterisation

Risk characterisation is the qualitative and/or quantitative estimation of the probability of occurrence and severity of known or potential adverse health effects in a given population based on hazard identification, hazard characterisation/dose–response, and exposure assessment (Lammerding, 1997). In risk characterisation all results of the former steps are integrated. All inaccuracies from the former steps accumulate in this integration. Furthermore, in this step the overall attendant uncertainty must be determined, using knowledge of the uncertainty in the statistical distributions of all factors. These distributions or its parameters are however often not known. When one knows for example the mean and stochastic distribution of all factors one can perform a Monte-Carlo simulation to determine the overall uncertainty. In a Monte-Carlo simulation a large number (for example 10.000) of simulations is carried out where, for each simulation, for every input factor a sample is drawn from its distribution. The distribution in the outcome of the risk calculation then gives a realistic probability distribution of the risk. For this, one needs to know the stochastic distribution of all input factors. When one does not know the distribution it is worth less, let alone when one does even not know the mean, which will be the case in many practical situations. A Monte-Carlo analysis then can be used as a type of sensitivity study, to find the most relevant effects, but cannot be used to determine a realistic uncertainty.

3.5. Sensitivity analysis

Three stages of sensitivity analysis will be presented in this paper, deterministic sensitivity, worst-case sensitivity and stochastic analysis, together with characteristic numbers for ranking.

If a stochastic analysis is carried out for all input factors, it is difficult to interpret the results and it takes much effort to select the distribution of all factors. Therefore it is of merit to select relevant stages and factors before the stochastic analysis.

3.5.1. Characterisation of the main determinants of risk

For a first selection of the most relevant determinants of the risk, a step characteristic *SC* can be used:

$$SC_k = \log\left(\frac{N_k}{N_{k-1}}\right) = \mu_k t_k / \ln(10) \quad (1)$$

with N_k the number of organisms, μ_k the specific growth rate and t_k the time in stage *k*.

The deterministic analysis uses the *SC* value to determine the sensitivity of the exposure estimate to the various process steps. Clearly, this results in an expression of the order of magnitudes of the impor-

tance of various process stages, enabling selection of relevant stages.

Risk is the product of the probability of contamination (P_c), the probability of survival (P_s) and the probability of illness (P_i). For the probability of survival it should be noted that if the inactivation results in less than 1 CFU product^{-1}, this probability starts to become of relevance (Table 1), and the number after inactivation starts at 1 per product (with a probability of P_s). So if the number after inactivation remains larger than 1 per product, the probability of survival is one. If inactivation results in a number smaller than 1 per product ($1/x$ per product) 1 out of x products contains one organism (a product cannot contain 0.1 organisms). This discontinuity is something which should be taken into account in calculations. Furthermore it is informative to compare the order of magnitude of P_c, P_s and P_i.

3.5.2. Worst case sensitivity

The worst-case analysis calculates $SC_{\text{worst-case}}$ for every worst-case input factor or possible extreme situation, to find risk-determining process steps in worst-case situations. The factor sensitivity (FS) shows the relevance of variations of a factor for each process step:

$$FS_k = \log\left(\frac{N_k(\text{extreme})}{N_k(\text{average})}\right) = SC_{k,\text{ worst-case}} - SC_{k,\text{ normal}} \quad (2)$$

High FS values mean high sensitivity to variations, and show that changes of factors in process steps have profound effects on N. For a first analysis every effect smaller than a factor 10 ($\log<1$) can be neglected, in order to search for the factors influencing risks mainly. After a step-by-step analysis, one can also check the worst-case scenario for factors in combination. This will probably be an unrealistic scenario, but might detect 'synergistic' effects between factors which can be overlooked by the step-by-step analysis. If the concentration ≥ 1 CFU product^{-1} throughout the process, ΣFS can also be estimated as:

$$\sum FS_k = \log\left(\frac{N_{\text{worst-case}}}{N_{\text{normal}}}\right) = \Delta\log(N) \quad (3)$$

The step-by-step analysis is however more useful as a start to clearly and simply detect main aspects. The worst-case sensitivity analysis is supplementary to the deterministic analysis, by giving extra information on the sensitivity of the process steps to varying process factors. In brief, the results of the deterministic and worst-case analysis consist of risk-determining and factor-sensitive process steps. It is sensible to study these steps stochastically.

Table 1
Number per product and probability of surviving organism(s) after various reductions

Reduction:	–	10	10^2	10^3	10^4	10^5
N (per product):	100	10	1	1	1	1
P_s[a]	1	1	1	0.1	0.01	0.001

[a] P_s is the probability of surviving organism(s) in the product.

3.5.3. Stochastic analysis

In a more detailed analysis the variations, by variability and uncertainty, of the risk-determining factors are described by frequency distributions. The Spearman rank correlation coefficient (*SRN*, Spearman Rank correlation coefficient for the number) is used to express the relevance of a varying factor for N (the number of organisms, i.e. the exposure).

$$SRN = 1 - \frac{6\sum_{i=1}^{n}(N_i - p_i)^2}{n(n^2-1)}$$

with n, the number of simulations; N_i, the rank number of N at the ith simulation; p_i the rank number of the factor under study at the ith simulation. Since the rank is used, the use of N or $\log(N)$ will yield the same result. *SRN* varies between -1 and 1. The closer $|SRN|$ is to 1, the higher the correlation, and thus the more important the factor is for the variability in the production process. In the same manner, an *SRP* value can be calculated that determines the correlation with the probability of survival for processes where the inactivation is such that the number is reduced below one per product. It should be noted that these correlations are correlations between the *variability* of the output and *variability* of the input factor, meaning that if a high correlation is found it can be concluded that the factor is largely responsible for the variability of the output. This will often be the case for factors that are

also, but not necessarily, determining the level of the output.

It is not always necessary to take statistical distributions into account for all aspects, since not all will be quantitatively important and those that are not important might confuse the interpretation of the results. Therefore, it is sensible to focus only on the selected steps and factors determined in the first two analyses.

In some cases it can be useful to distinguish uncertainty (lack of knowledge, data, model inaccuracy) and variability (biological, physical, chemical). Uncertainty can be reduced often by more research (more data, more insight, specific data), variability often by technological solutions (better process control, more standardisation).

4. Example for *Salmonella* on chicken

4.1. Deterministic sensitivity analysis

If the organism parameters of Table 2 are used for *Salmonella* and an example process as outlined in Table 3, main steps can be seen graphically (Fig. 2) and by the *SC* value (Table 3). The process is simply an example to show the virtues of the sensitivity analysis. The models used to calculate growth and inactivation rates are the Cardinal Temperature and pH Model (Rosso et al., 1995) and the *D*, *z* model. Step 7 is found to be quantitatively the most important (Table 3). The inactivation results in less than 1 CFU product^{-1}, so the probability of survival (P_5) starts to become of relevance, and the number after inactivation starts at 1 per product (with a probability of P_s). In this example, $\log(P_s) = \log(N_0) + \Sigma SC_{\text{through cooking}} = -4.86$, so $P_s = 1.4\text{E}-5$, one in 73 000 products contains one CFU after heat treatment.

4.2. Worst-case determination

For every possible factor of the quantitative determination, the worst-case situation can be determined and its quantitative effect estimated. In this manner, main determining steps can be identified, but in this case for exceptional conditions. More importantly, however, steps are detected where even a worst-case estimate gives no relevant change in numbers. This helps to focus on relevant details. In Table 4 this is worked out for the example of Table 3. The effect is quantified considering a longer presence in the slaughtering line due to machine problems, slower cooling due to bad conditioning, abuse temperature storage1, wrong cooking tempera-

Table 2
Organism parameters of *Salmonella* (ICMSF, 1996; Wijtzes et al., 1998)

T_{min}	T_{opt}	T_{max}	pH_{min}	pH_{opt}	pH_{max}	a_{wmin}	μ_{opt} (h^{-1})	D_{ref} (min)	T_{ref}	z
5.2	37	46.2	3.8	7	9.5	0.94	1.65	2	60	10

Table 3
Process steps of a chicken product with Step Characteristic (*SC*) and predicted growth. Bold values highlight relevant effects

Step		t (h)	T	$SC = \mu^* t/\ln(10)$	Log(N)
	Initial				2
1	Slaughter	1	37	0.72	2.72
2	Cool 1	0.25	30	0.15	2.86
3	Cool 2	0.25	20	0.06	2.92
4	Cool 3	0.25	15	0.03	2.95
5	Cool 4	0.25	10	0.01	2.96
6	Storage 1	100	6	0.08	3.04
7	Cooking (5 min)	0.0833	65	**−7.90**	−4.86
8	Storage 2	24	10	0.69	0.69[a]

[a] The number after inactivation started at 1 CFU per product.

Table 4
Worst-case analysis showing Step Characteristic (*SC*) and Factor Sensitivity (*FS*). Bold values highlight relevant effects

Step		*t* (h) initial	*T* initial	*t* (h) worst	*T* worst	$SC_{worst\text{-}case}$	*FS*
1	Slaughter	1	37	2	–	**1.43**	0.72
2	Cool 1	0.25	30	1	–	0.58	0.44
3	Cool 2	0.25	20	1	–	0.25	0.19
4	Cool 3	0.25	15	1	–	0.11	0.09
5	Cool 4	0.25	10	1	–	0.03	0.02
6	Storage1	100	6	–	10	**2.87**	**2.79**
7	Cooking	0.0833	65	–	60	**−2.50**	**5.40**
8	Storage2	24	10	–	12	**1.36**	0.67

ture, and storage2 temperature abuse. Table 4 shows $SC_{worst\text{-}case}$ values; relevant changes in microbial load during the production process occur during slaughter and storage1, as well as heat treatment and storage2. The output sensitivity, *FS*, shows that the process steps storage1 and cooking are sensitive to variations in the factors. Cooking was already detected in the deterministic analysis. This example shows that steps may be irrelevant in the deterministic analysis and become important in the worst-case analysis. Also a step can be relevant in the deterministic analysis, but show no relevant deviation in the worst-case situations, although often the same stages will be detected as being important. For the main determining stages detected in both of the two phases the stochastic analysis is relevant. One can focus now especially on the stochastic distribution of these factors in phase 3, i.e. the stochastic analysis.

4.3. Stochastic analysis

As an example, variations in the factors of the steps highlighted in Table 4 were described by the triangle distribution (Triang): Slaughter, storage1, cooking, and storage2. The minimum, most likely, and maximum values presented in Table 5 are example values. Triang is used as a rough modelling tool where the range (a–c) and the most likely value (b) can be estimated; it is flexible in shape and it has intuitive parameters (Vose, 1996). After entering the distributions and related values, exposure assessment was simulated 10000 times for the production process in Table 3.

The *SRN* value is a measure of the relevance of a parameter's variability, instead of being a measure of the relevance of a parameter's numerical value. *SRN* values for stages 1, 6 and 7 are rather low, meaning that the variation hardly affects the variation in the estimated number of organisms. In this case, clearly the number is determined by the last step storage2 since, due to the heating process, only in limited cases is a survivor found and, if so, growth will only be determined by the last step. So the variability in the number is only determined by the variability in the last step (growth after survival). Comparing the *SRP* values, which give the correlation between the variability of the input factors and the probability of survival, allows the conclusion that the probability of survival depends on the number before heating and also on the steps before. Of course, it is not correlated with the process step after heating. The variability of the temperature of cooking is the main

Table 5
Values for the parameters of the triangle distribution, for various process steps and the Spearman Rank correlation coefficient for the number (*SRN*) and for the probability of survival (*SRP*)

Step		Parameter *p*	Minimum	Most likely	Maximum	*SRN*[a]	*SRP*[a]
1	Slaughter	Time (h)	0.5	1	2	0.039	0.11
6	Storage1	Temperature (°C)	5	6	10	0.11	0.27
7	Cooking	Temperature (°C)	60	65	66	−0.18	**−0.94**
8	Storage2	Temperature (°C)	9	10	12	**0.89**	0.0042

[a] *SRN* is Spearman Rank correlation for the exposure *N*; *SRP* for the probability of survival. These are approximate values (at every run results are slightly different, the mean values of 5 runs with 2000 simulations are presented).

determinant of the variability of survival. It is also of value to examine all correlations graphically.

The deterministic and worst-case analyses clearly showed the relevance of cooking temperature as an important risk determining step. To achieve a safe food process, this temperature should be well controlled. A second result of the worst-case analysis is the relevance of variations in slaughter, storage1, and storage2. The stochastic analysis then showed that the variability in exposure estimates is determined mainly by storage2. The variations in other processes (slaughter, storage1, cooking) determine the probability of survival, of which the cooking temperature is clearly the most important.

5. Conclusions

Many different factors impact on the extend to which a pathogen poses a risk to consumers and some of these factors are more important than others: Whereas some factors may have only 30% influence, others are in the order of 10^6. Therefore, it is important to focus risk assessments initially on the main factors. For this purpose, many sources of information (experts, databases, modelling programs, literature) can be used critically. In order not to get overwhelmed with detail in the first instance, one should start with a simple deterministic sensitivity analysis. Such an analysis will give insight to the important processes and phenomena and will show main-determining steps and relevant aspects. However, using only this type of analysis, one can overlook exceptional but very relevant cases. Therefore, two other types of sensitivity analyses are presented, in addition to a deterministic sensitivity analysis: worst-case and stochastic.

The deterministic analysis and worst-case analysis identify the factors that mainly determine the extent of the risk and that, thus, represent CCP's in the process. The stochastic analysis then investigates these main determinants of risk in detail, and shows clearly the (in)accuracy in the risk estimate. Employing, for example, rank order correlation the main determinants of variation of risk can be determined to subsequently give improvements in the quantification of risk.

The methods presented here quantitatively support a risk assessment. They can be helpful to determine hazards, determine main phenomena quantitatively important for risk, calculate the effect of changes in the process, and enable a wide range of operating strategies to be evaluated. The simulation procedures do not yield absolute predictions, but orders of magnitude. The results, however, can be used as the basis for decisions and to determine which stages or processes require more detailed analysis. The simulations show that in certain cases, one should concentrate on the microorganism's growth or inactivation parameters, in other cases on the rate of cooling of the food product or on the variability of the composition. Also the accuracy of the estimate may be totally determined by the dose–response relation in some cases and the variability in host susceptibility or organism virulence in others. Therefore, it is important to determine for every case which aspects are of relevance and which of these have the largest impact on the risk estimate. For this a quantitative determination procedure with different types of sensitivity analyses is useful. In time, new information will become available, therefore continuous updating will be necessary. This will gradually improve the confidence in the decisions. The predictions are based on objective qualitative and quantitative information. Even with stochastic output, one should never solely rely on the models used. They should be used to support decision making and not to escape from responsibility. Structured and transparent risk analysis is open for criticism. This criticism should not be used to condemn the analysis, but to improve the results.

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