Supplement

Food Safety Objective Approach for Controlling *Clostridium botulinum* Growth and Toxin Production in Commercially Sterile Foods

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ABSTRACT

As existing technologies are refined and novel microbial inactivation technologies are developed, there is a growing need for a metric that can be used to judge equivalent levels of hazard control stringency to ensure food safety of commercially sterile foods. A food safety objective (FSO) is an output-oriented metric that designates the maximum level of a hazard (e.g., the pathogenic microorganism or toxin) tolerated in a food at the end of the food supply chain at the moment of consumption without specifying by which measures the hazard level is controlled. Using a risk-based approach, when the total outcome of controlling initial levels (H_0), reducing levels (ΣR), and preventing an increase in levels (ΣI) is less than or equal to the target FSO, the product is considered safe. A cross-disciplinary international consortium of specialists from industry, academia, and government was organized with the objective of developing a document to illustrate the FSO approach for controlling *Clostridium botulinum* toxin in commercially sterile foods. This article outlines the general principles of an FSO risk management framework for controlling *C. botulinum* growth and toxin production in commercially sterile foods. Topics include historical approaches to establishing commercial sterility; a perspective on the establishment of an appropriate target FSO; a discussion of control of initial levels, reduction of levels, and prevention of an increase in levels of the hazard; and deterministic and stochastic examples that illustrate the impact that various control measure combinations have on the safety of well-established commercially sterile products and the ways in which variability all levels of control can heavily influence estimates in the FSO risk management framework. This risk-based framework should encourage development of innovative technologies that result in microbial safety levels equivalent to those achieved with traditional processing methods.

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The minimum of a 12-log reduction of *Clostridium botulinum,* the basis for determining a safe process for thermally processed commercially sterile food, has not changed in more than 90 years, but processing technologies that could replace traditional thermal processes have developed rapidly in recent years. According to the Codex Alimentarius Commission *(21),* '''Commercial sterility of thermally processed food' means . . . to render the food free from microorganisms capable of growing in the food at normal non-refrigerated conditions at which the food is likely to be held during distribution and storage.'' Often the treatment necessary to render a food product commercially sterile does not target a defined safety level but instead targets the prevention of spoilage when the food product is held under normal storage conditions. As a result, there is a great deal of variability among processes applied to

commercially sterile products around the world, even among very similar products. As global trade of commercially sterile foods increases, this variability creates barriers to trade because technological capabilities differ among countries and processing capabilities differ among food companies within the same country. Various food legislation and regulations also can produce serious trade obstacles *(49)*. The World Health Organization (WHO)–Food and Agriculture Organization Food Standards Programme *(22)*, which developed microbiological criteria for foods in international trade, was established as a direct result of conflict between national food legislation and general requirements of world food markets. Since the establishment of this program, governments around the world have adopted a risk-based approach to food safety management, and this approach is strongly supported by intergovernmental organizations *(114)*.

Internationally, there has been a move toward outcome-based food standards. Outcome-based standards designate the maximum level of a hazard (e.g., the microorganism or the toxin it produces) allowed in a food at the end of the food supply chain at the moment of consumption *(37, 38)*. Thus, outcome-based food standards allow empirical measurement of process performance with risk analysis. In response to this movement and global food safety concerns, the International Commission on Microbiological Specifications for Foods (ICMSF) developed a new risk management framework based on the principles of a food safety objective (FSO), a concept later adopted by the Codex Alimentarius Commission *(22)*. An FSO is defined as ''the maximum frequency and/or concentration of a hazard in a food at the time of consumption that provides or contributes to the appropriate level of protection (ALOP)'' *(49)*. The ALOP is used to describe the risk level that is deemed an appropriate or tolerable public health goal (PHG) and must conform with regulatory policies and public opinion. The definition of a ''reasonable'' ALOP differs among countries, and acceptable risk may be culturally defined. FSOs help translate the health risks associated with particular hazards into goals that are more easily definable at the manufacturing level and across trade lines. The FSO is then used to derive related metrics upstream in the food supply chain to achieve the same endpoint levels. In this way, the FSO approach establishes a metric that can be used to judge equivalent levels of hazard control stringency.

Historically, major advances in consumer protection have resulted from the development and implementation of targeted control measures at one or more steps along the food chain. Control measures are the actions or activities used to prevent, eliminate, or reduce a food safety hazard to an ALOP. These measures generally fall into three categories: controlling initial levels (H_0), reducing levels (ΣR), and preventing an increase in levels (ΣI). The outcomes of these control measures are defined as performance criteria. When establishing a performance objective (PO) or criterion, consideration must be given to the initial level of a hazard, changes to levels that occur as a result of control measures instituted during production, and changes to levels that occur during storage, distribution, preparation, and use of the

product. A PO is preferably less than but at least equal to the FSO and can be expressed by equation 1 *(49)*:

$$H_0 - \sum R + \sum I \leq \text{FSO} \qquad (1)$$

where H_0, ΣR, ΣI, and FSO are expressed in log units. For a canned food, the PO and the FSO can be the same because no increase in the hazard can occur before consumption.

The purpose of this article is to introduce a structured approach to managing the safety of ''commercially sterile'' foods at an ALOP. In most cases, a commercially sterile product requires refrigeration after opening by the consumer. Consequently, this discussion focuses on the safety of the final product as it pertains to the protection of public health. It does not address the impact of processing on nutritive or sensory quality of product nor the loss of product as a result of growth of nonpathogenic mesophilic or thermophilic spore-forming bacteria, a process commonly referred to as economic spoilage. However, the principles outlined in this document can be used to design a process that targets mesophilic and thermophilic organisms while delivering a commercially sterile product.

The National Center for Food Safety and Technology at the Institute for Food Safety and Health (IFSH; Illinois Institute of Technology, Bedford Park) organized a cross-disciplinary international consortium of specialists from industry, academia, and government to develop a document to illustrate how the Codex risk management framework could be used for controlling *C. botulinum* growth and toxin production in commercially sterile foods. Members of the steering committee and their affiliations are listed in Table 1. Contributing authors from each of the four committees are listed in Table 2.

This document outlines historical approaches to establishing commercial sterility, explores methods for setting an ALOP and FSO for commercially sterile foods, and describes the principles of risk-based process development. Well-established products in the marketplace have been used as examples to illustrate how these products, developed over a long period of time, fit into this risk management framework. Canned beans, shelf-stable canned cured luncheon meat, shelf-stable pesto, and shelf-stable pasteurized processed cheese spread were chosen as example food products to reflect the diversity of commercially sterile foods and the various approaches to establishing commercial sterility of shelf-stable products. For example, the commercial sterility of canned beans relies primarily on thermal processing, shelf-stable canned cured luncheon meat relies on a combination of both thermal processing and formulation of the product to establish a state of commercial sterility, and commercial sterility of shelf-stable processed cheese spread and pesto sauce relies primarily on product formulation. For each example given, the product bioburden, the reduction in the level of the hazard, and the increase in the level of the hazard during or after processing are presented; however, the ALOP and FSO are left to be established by a competent authority in keeping with regulatory policies and public opinion.

Commercial sterility: food industry definition. Not all of the microorganisms in the product must be destroyed for a product to be considered commercially sterile. Rather,

TABLE 1. *Steering committee members*

Name	Affiliation[a]
Nathan M. Anderson, Ph.D. (Chair)	IFSH, NCFST, FDA
Martin B. Cole, Ph.D.	IFSH, NCFST, IIT (currently with CSIRO)
John W. Larkin, Ph.D.	IFSH, NCFST, FDA
Cynthia M. Stewart, Ph.D.	IFSH, NCFST, IIT (currently with PepsiCo)
Guy E. Skinner, Ph.D.	IFSH, NCFST, FDA
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Alfredo Rodriguez, Ph.D.	IFSH, NCFST, IIT (currently with PQS Technologies, LLC)
Larry Keener, Ph.D.	International Product Safety Consultants
Richard Whiting, Ph.D.	FDA (currently with Exponent)
Leon Gorris, Ph.D.	Unilever
Robert Buchanan, Ph.D.	FDA (currently with University of Maryland)
Paul Hall, Ph.D.	ConAgra (currently with AIV Microbiology & Food Safety)

[a] CSIRO, Commonwealth Scientific and Industrial Research Organisation, Australia; FDA, U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition; IFST, Institute for Food Safety and Health; IIT, Illinois Institute of Technology; NCFST, National Center for Food Safety & Technology.

the food must be free of microorganisms ''capable of growing'' under normal nonrefrigerated conditions of storage and distribution *(21)*. Many control measures or combinations of control measures can be used to control the growth of microorganisms remaining in the finished product. No distinction between pathogenic and nonpathogenic organisms is made in this definition of commercial sterility.

TABLE 2. *Contributing authors from the four committees*

Committee	Affiliation[a]
FSO	
Richard Whiting, Ph.D. (Chair)	FDA (currently with Exponent)
Leon Gorris, Ph.D.	Unilever
Martin B. Cole, Ph.D.	IFSH, NCFST, IIT (currently with CSIRO)
Robert Buchanan, Ph.D.	FDA (currently with University of Maryland)
Peter McClure, Ph.D.	Unilever
Clive Blackburn, Ph.D.	Unilever
Alejandro Amezquita, Ph.D.	Unilever
H_0	
Guy E. Skinner, Ph.D. (Chair)	IFSH, NCFST, FDA
R. Bruce Tompkin, Ph.D.	Food safety consultant
Donald Zink, Ph.D.	FDA
David Legan, Ph.D	Kraft
Dan Brown, Ph.D	Hormel (retired)
Sam Palumbo, Ph.D.	IFSH, NCFST, IIT (deceased)
ΣR	
Alfredo Rodriguez, Ph.D. (Chair)	IFSH, NCFST, IIT (currently with PQS Technologies, LLC)
John W. Larkin, Ph.D.	IFSH, NCFST, FDA
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Tatiana Koutchma, Ph.D.	IFSH, NCFST, IIT (currently with Agriculture Canada)
George Evancho, Ph.D.	Campbell Soup Company (currently with University of Maryland)
ΣI	
Raymond Carroll (Chair)	Campbell Soup Company (currently a food safety consultant)
Nathan M. Anderson, Ph.D.	IFSH, NCFST, FDA
Larry Keener, Ph.D.	International Product Safety Consultants
Cynthia M. Stewart, Ph.D.	IFSH, NCFST, IIT (currently with PepsiCo)
Robin Kalinowski	IFSH, NCFST, IIT (currently with Mars Petcare)
Paul Hall, Ph.D.	ConAgra (currently with AIV Microbiology & Food Safety)

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Regulations in the United States. In the United States, food safety is a shared responsibility. Several departments of the U.S. government share jurisdiction in ensuring the safety of the American food supply. The predominant U.S. agencies that regulate commercially sterile foods are the U.S. Food and Drug Administration (FDA) of the U.S. Department of Health and Human Services and the Food Safety and Inspection Service (FSIS) of the U.S. Department of Agriculture (USDA). The FDA regulates all seafood and nonmeat products and foods that contain less than 3% raw or 2% cooked meat or poultry ingredients. The USDA regulates products that contain larger amounts of meat and/or poultry.

The FSIS regulation that defines commercial sterility and shelf stability (9 CFR 318.300(u)) *(103)* uses language similar to that used by Codex:

> Shelf stability. The condition achieved by application of heat, sufficient, alone or in combination with other ingredients and/or treatments, to render the product free of microorganisms capable of growing in the product at nonrefrigerated conditions (over 50°F or 10°C) at which the product is intended to be held during distribution and storage. Shelf stability and shelf stable are synonymous with commercial sterility and commercially sterile, respectively.

The FDA expectation of commercial sterility is that the process used will eliminate microbial spoilage and any potential public health hazard under normal storage conditions. Therefore, the FDA definition of commercial sterility distinctly adds that ''all viable microorganisms (including spores) of public health significance'' must also be destroyed or controlled from reproduction ''by the application of heat'' or ''by the control of water activity and the application of heat.'' The full U.S. Code of Federal Regulations citation for commercial sterility (21 CFR 113.3(e)) *(107)* is as follows:

(1) ''Commercial sterility'' of thermally processed food means the condition achieved—
   (i) by the application of heat which renders the food free of—
     (a) microorganisms capable of reproducing in the food under normal nonrefrigerated conditions of storage and distribution; and
     (b) viable microorganisms (including spores) of public health significance; or
   (ii) by the control of water activity and the application of heat, which renders the food free of microorganisms capable of reproducing in the food under normal nonrefrigerated conditions of storage and distribution.
(2) ''Commercial sterility'' of equipment and containers used for aseptic processing and packaging of food means the condition achieved by application of heat, chemical sterilant(s), or other appropriate treatment that renders the equipment and containers free of viable microorganisms having public health significance, as well as microorganisms of nonhealth significance, capable of reproducing in the food under normal nonrefrigerated conditions of storage and distribution.

Both the FDA and the USDA have specific regulations for shelf-stable foods when the final food product is defined as a low-acid food product. The FDA *(108)* defines a low-acid canned food product as follows: ''Low-acid foods means any foods, other than alcoholic beverages, with a finished equilibrium pH greater than 4.6 and a water activity (a_w) greater than 0.85. Tomatoes and tomato products having a finished equilibrium pH less than 4.7 are not classed as low-acid foods'' (21 CFR 133.3(n)), whereas the FSIS defines a canned food as ''a meat food product with a water activity above 0.85 which receives a thermal process either before or after being packed in a hermetically sealed container'' (9 CFR 318.300(d)) *(101)* and a low-acid food as ''a canned product in which any component has a pH value above 4.6'' (9 CFR 318.300(m)) *(102)*.

Another important element of the concept of commercial sterility is that the product must be placed in a hermetically sealed container and handled in a manner that prevents the product from becoming recontaminated. The understanding is that the process used to make the product safe is sufficient to generate a commercially sterile product and that the product will be kept sterile until the consumer opens the package. After opening, the food may require refrigeration and may have a limited shelf life.

International regulations. Many countries around the globe have their own national food control systems. The European Union, a confederation of 27 member countries, has begun harmonizing the food control systems used in Europe. The European Parliament and the Council of the European Union regulation on the hygiene of foodstuffs *(31)* lists requirements for heat treated foods in hermetically sealed containers that are nonspecific, noting only that ''the process used should conform to an internationally recognised standard (for example, pasteurisation, ultra high temperature or sterilisation).'' The Scientific Panel on Biological Hazards of the European Food Safety Authority (EFSA) *(32)* issued an opinion specifically on *Clostridium* spp. in foodstuffs, which includes a section on control of *C. botulinum* by heat treatment that refers to the application of the ''botulinum cook'':

> The application of the ''*botulinum* cook'' is defined as equivalent to 3 minutes heating at 121°C. This value is also the F_o value or the process value. This heating regime is used for low acid canned food products and results in a 12 $\log_{10}$ units reduction in numbers of spores.
>
> The F_o value required for canned food products is equivalent to 12-decimal reductions of proteolytic *C. botulinum* spores. Using the highest known D-values (0.25 min at 121 °C), the F_o is therefore equal to $12 \times 0.25 = 3$. This is the so-called 12 D-concept designed to reduce the bacterial load of one billion spores in each of 1000 cans to 1 spore in a thousand cans.

In Europe, a standard that has been used within the food industry for the tolerable rate of spoilage is one spore per 10,000 units *(12)*.

Sterility: pharmaceutical industry definition. Medical drugs and devices, by their very nature, must be free of any potential microbial hazard. For this reason, the FDA

expectation is that each drug or device must be treated to achieve a condition of sterility, which means a complete absence of viable microorganisms. As explained within the *U.S. Pharmacopeia–National Formulary (110),* sterility must be expressed as a probability of finding a contaminated unit:

Within the strictest definition of sterility, a specimen would be deemed sterile only when there is complete absence of viable microorganisms from it. However, this absolute definition cannot currently be applied to an entire lot of finished compendial articles because of limitations in testing. Absolute sterility cannot be practically demonstrated without complete destruction of every finished article. The sterility of a lot purported to be sterile is therefore defined in probabilistic terms, where the likelihood of a contaminated unit or article is acceptably remote. Such a state of sterility assurance can be established only through the use of adequate sterilization cycles and subsequent aseptic processing, if any, under appropriate current good manufacturing practice, and not by reliance solely on sterility testing.

A specific sterility assurance level, i.e., the likelihood of a potentially hazardous product becoming nonsterile before the end of its life cycle, is required to validate the ability of a system to deliver a sterile product. The FDA set a sterility assurance level for each process cycle of one nonsterile unit per every 1 million units produced. The probability of a nonsterile unit (PNSU) frequently is used to describe the ratio of the probability of a single microorganism surviving in a specific number of units *(74).*

Ultimately, cycle specifications for such sterilization methods should be based on the delivery of adequate lethality to the locations in the material that are slowest to heat. A sterility assurance level of 10^{-6} or better should be demonstrated for a sterilization process *(106).*

By definition, a sterile drug must have a PNSU of 10^{-6} per unit. When the PNSU standard is applied, the final level of a surviving microbial hazard defines product safety, and therefore the processor must ensure that the initial level of contamination is at or below the expected initial level.

Historical perspective on risk assessment. The kinetics of chemical and moist-heat inactivation of a microorganism were first defined by the pseudo first-order Chick-Watson model *(20).* The foundation for process calculations was laid by Bigelow et al. *(8)* when they described the original general method, which required graphical integration of the lethal rate of the temperature profile over the total heating time to arrive at a sterilization value, a measure of heat treatment severity. It was not until the 1950s that the logarithmic nature of microbial destruction and the *D*-value was added to the process establishment procedure of Ball and Olson *(6).* The Ball formula method was the first to utilize the concept of thermal death time (TDT) to calculate process lethality *(5).* TDT is the time necessary to destroy a microbial population at a specific temperature *(7).* ''Death'' generally indicates the failure of a given microbial population to grow in the subculture medium after the heat treatment *(78).* TDT data are obtained by subjecting a microbial population to a series of heat treatments at a given temperature and testing for survivors. These data can then be used to calculate the *z*-value, the temperature range that results in a factor of 10 change in the decimal reduction time (*D*-value). The *D*-value is the heating time required for a 90% reduction in the initial microbial population. The TDT depends on the initial microbial load, whereas the *D*-value does not.

Today's low-acid canned food processing specialists have developed a process design based on the physical attributes of the process (e.g., heating rates of the food product, product composition, and container size), the target organism of concern, the necessary treatment dose, and the kinetic parameters used to describe the destructive behavior of the organism of concern (i.e., *D*- and *z*-values). The food industry has tended to use processes that result in a specific log reduction of a target microorganism instead of a specific endpoint. When the food product supports the growth of *C. botulinum,* the process authority often will target a process treatment that delivers a minimum 12-log reduction of *C. botulinum,* which would also prevent spoilage under normal storage conditions. The processing effect of a 12-log inactivation, or the ''*botulinum* cook,'' is based on the extrapolated values (121.1°C) of data obtained by Esty and Meyer *(33),* who studied the resistance of 60 billion of the three most heat-resistant *C. botulinum* spores in phosphate buffer at 120°C and established a *z*-value of 10°C. The log-reduction process design assumes that the initial level of the contaminant will never go above a predetermined maximum expected level and that to deliver a specific level of safety a known log reduction of the organism of concern must be delivered by the process. There is generally considered to be an inherent safety factor in the assumption that there will be 60 billion resistant *C. botulinum* spores present. The application of such a large safety factor may compensate for an occasional can of food that heats more slowly than anticipated and for the occasional mechanical or human error that occurs in production *(72).* Application of the *D*-value of Stumbo *(92)* makes it possible to use a practical number of spores for TDTs (1 million per container is usual) and to calculate a commercial $F_{121°C}$ value (the unit of measurement used to compare the relative sterilizing effects of different procedures equal to 1 min at 121.1°C) relative to 60 billion spores by multiplying the *D*-value by 12. The *D*-value used for *C. botulinum* at 121.1°C is 0.21 to 0.25 min. Thus, the typical target treatment for a *botulinum* cook is 2.5 to 3.0 min at 121.1°C. The food industry as a whole has found a 12-log reduction of *C. botulinum* to be an acceptable target level of public health risk that can be used to design a sterilization process. However, a frequent assumption and common misconception is that the FDA requires a 12-log reduction of *C. botulinum* for low-acid canned foods.

To specify an endpoint and still satisfy the requirement of commercial sterility when designing sterilization processes, Pflug *(73)* adapted the PNSU concept to food applications by identifying a target endpoint for each of three preservation conditions: public health, preservation from growth of mesophilic organisms, and preservation from growth of thermophilic organisms. Pflug's recommended levels of endpoint probability of a surviving cell capable of growth per container are as follows:

- Public health (*C. botulinum*) $= 10^{-9}$
- Preservation from mesophilic spoilage $= 10^{-6}$
- Preservation from thermophilic spoilage $= 10^{-2}$ for normal storage
- Preservation from thermophilic spoilage $= 10^{-6}$ for elevated storage temperatures

The food industry has not adopted this approach for establishing sterilization processes.

Process establishment and validation of new technologies. Historically, *C. botulinum* has been the microorganism of public health concern considered when developing preservation methods for shelf-stable low-acid foods; however, this organism may not be an appropriate standard for all future processing technologies. Although in some cases past experience can be immediately transferred to a new or novel processing technology such as UV light, all assumptions associated with past experimental procedures must be verified for their applicability to the new application. The procedure of establishing a process and validating its effectiveness is dependent on the processing system (e.g., heat, high pressure, or UV light) and the food product. New technologies may involve different mechanisms of inactivation, which means that *C. botulinum* may not be the most resistant microorganism of public health concern. Therefore, identification of the appropriate target organism of concern is critical. Once identified, the destructive behavior of the organism of concern for public health must be evaluated and an appropriate target endpoint selected. A sufficiently verified experimental approach must be used to collect information critical to understanding process delivery and achievement of the target endpoint. A general approach to the establishment of processing technologies includes the following elements:

- Identification of the organism of concern
- Identification and selection of the appropriate target endpoint
- Development of a conservative estimation of the ability of the process to consistently deliver the target endpoint
- Quantitative validation (microbiological or mathematical) of the lethal treatment delivered
- A list of the critical factors and procedures necessary to control and ensure the delivery of the required process

Although a discussion of all potential hurdles that must be controlled when obtaining processing data is outside the scope of this article, a number of general steps recommended when validating and certifying a sterilization process have been outlined by the U.S. Pharmacopeia *(110)*:

1. Establish that the process equipment is capable of operating within the required parameters.
2. Demonstrate that the critical control equipment and instrumentation are capable of operating within the prescribed parameters for the process equipment.
3. Perform replicate cycles representing the required operational range of the equipment and employing actual or simulated product.

4. Demonstrate that the processes have been carried out within the prescribed protocol limits and finally that the probability of microbial survival in the replicate processes completed is not greater than the prescribed limits.
5. Monitor the validated process during routine operation. Periodically as needed, requalify and recertify the equipment.
6. Complete the protocols, and document steps 1 through 4 above.

Framework for discussing equivalence of technologies. As existing technologies are refined and novel microbial inactivation technologies are developed, there is a growing need for a metric that can be used to judge equivalent levels of hazard control stringency to ensure the safety of commercially sterile foods. The ability to understand the equivalent safety of a thermal process is important for commerce and for compliance with associated regulatory requirements, both domestic and international. For a new or novel sterilization technology such as high-pressure processing, the means for assessing whether it provides an equivalent level of protection relative to traditional, well-understood thermal processes are not obvious. In the context of this discussion, for two processes to have an equivalent level of protection, each method must result in an equal probability that a single unit of commercially sterile food would contain *C. botulinum* toxin. The sterilization value enables the thermal processing industry to easily relate the lethality of different thermal processes by using a common reference temperature and incorporating both the kinetics of the destruction of the organism of concern and the mathematics of heating in one simple term. The reference sterilization value, denoted F_o, represents the time for a given reduction of microbial spores with a *z*-value of $10°C$ at a temperature, *T,* of $121.1°C$. However, there may not always be an obvious way to assess different processes or even different commodities produced using the same processing system. The nature of a process may limit the ability to develop values equal to the sterilizing value used within the thermal processing industry. However, there needs to be a procedure by which the use of a risk assessment will result in a known level of safety for a process and thus will provide a means to establish equivalence between processes and products produced.

The food industry, which has a long history of producing safe thermally processed food products, has been able to

- identify the microorganism(s) of public health concern,
- identify the target treatment(s) needed to ensure public safety,
- understand the destruction kinetics necessary to evaluate a treatment,
- understand how products heat for each processing system, and
- generate principles on the relationships between the microorganism of public health concern and spoilage.

As the food industry has developed this understanding over the last century, food manufacturers also have developed a tendency to adopt more conservative approaches by using worst-case results in the procedure for establishing a thermal process. Pflug (73) pointed out that the original *C. botulinum* resistance data were obtained with spores grown from optimum growth medium and do not necessarily represent the type of resistance that might occur from spores occurring naturally. However, an expectation of increased resistance is good practice because several conditions may generate higher heat resistances, such as sporulation in the presence of divalent cations and at higher temperatures. There may be conditions in the external environment that lead to generation of spores with relatively high resistance; however, it is impractical to determine resistance of naturally occurring spores. A good example of a worst-case approach is use of data from the most heat-resistant strains, which probably are not representative of the wide variety of strains found in nature. The data of Esty and Meyer (33) indicate higher heat resistances in some foods, such as corn and spinach, and lower heat resistance in other foods, such as sweet potatoes, asparagus, and string beans, relative to the resistance in phosphate buffer.

RISK ASSESSMENT

Modern food safety systems, such as hazard analysis critical control point (HACCP) programs (64), are based on preventive strategies. The underlying principle of these control systems is that the stringency should be proportional to the likelihood of occurrence and severity of illness (i.e., risk to public health). Effective management of microbial food safety hazards requires identification of the hazards, assessment of risks associated with those hazards, and estimation of the effectiveness of control measures (49).

Historically, industry has relied on the expertise of process authorities to estimate the level of control needed to ensure commercial sterility and protect public health. This expertise, and the conservative approach that goes with it, has led to very reliable processes and an exceptional track record of safety in spite of recent foodborne illness outbreaks. However, the underlying scientific basis and rationale for decisions often were not documented or were proprietary in nature, which has led to great variability between processes applied to low-acid canned products around the world, even among very similar products.

An improved approach is the use of structured safety assessments, typically including broader scientific expertise and more formal consideration of available data and information (49). Quantitative risk assessment was introduced to systematically evaluate various factors that contribute to the risk associated with foodborne microbiological hazards. A risk assessment involves four steps: hazard identification, exposure assessment, hazard characterization, and risk characterization (23). This approach has been widely adopted and used by several expert panels called to address various food safety issues, including *Clostridium* spp. in foodstuffs (32).

Scope. Since the EFSA has already completed a thorough risk assessment of *C. botulinum* (32), the present article does not provide an exhaustive risk assessment but rather highlights some important aspects related to commercially sterile foods and the associated risks to public health from *C. botulinum*.

As alternative processes or new technologies are developed, other pathogens may exhibit greater resistance to the process than would *C. botulinum*. For example, *Bacillus cereus* is a ubiquitous, aerobic spore-forming pathogen. Blakistone et al. (9) reported that *B. cereus* was more resistant than *C. botulinum* to a 2% concentration of peroxyacetic acid solution. The decreased sensitivity of *B. cereus* to peroxyacetic acid raised concern about the efficacy of the sterilant for aseptic packaging of low-acid foods. Further, a 6D nonproteolytic *botulinum* cook is not adequate to inactivate *B. cereus*. Therefore, a thorough risk assessment must be conducted that takes into consideration the appropriate target microorganism(s) and type of food and processing system used that may contribute to the risk associated with that operation.

C. botulinum. Consumption of raw product contaminated with spores of *C. botulinum* does not cause botulism, except in infants and some immunocompromised populations. Botulism almost always occurs after ingestion of the neurotoxin formed when spores of *C. botulinum* germinate and multiply in a food. Outbreaks may occur after a processing failure or during formulation or are related to container integrity. A food may contain viable *C. botulinum* and still not be capable of supporting toxin production. Growth of *C. botulinum* can be prevented in foods, naturally or by design, by controlling pH and water activity, adding an inhibitory concentration of a preservative, or controlling two or more of these factors in combination (88).

Since the 1920s, significant changes have occurred in the commercial manufacturing of canned foods (e.g., the implementation of good manufacturing practices [GMPs]). Unreliable technologies that do not produce consistent levels of food safety tend to be discontinued or modified over time. This evolution has led to very reliable processes and an exceptional track record for safety, though an outbreak of botulism in industrially produced low-acid canned chili sauce occurred as recently as July 2007 (17). In spite of recent events, relatively few outbreak cases have been attributed to commercially produced foods intended to be commercially sterile.

Although botulism is a rare food illness, its mortality rate is high. The 962 botulism outbreaks recorded in the United States from 1899 to 1990 involved 2,320 cases and 1,036 deaths (14). Though identification and treatment of botulism have improved, death still occurs in 5 to 10% of cases of foodborne botulism (14). The most common source of these outbreaks was been home-canned foods prepared in an unsafe manner (16). However, the most common sources of botulism in the United States today are ethnic foods fermented by Alaskan natives (15). The WHO recorded 6,493 reported cases of botulism in Europe, Central Asia, and the Baltic region between 1996 and 2006. More than 80% of these cases were attributed to home-preserved vegetables (70). Attempts to transfer lessons from industrial
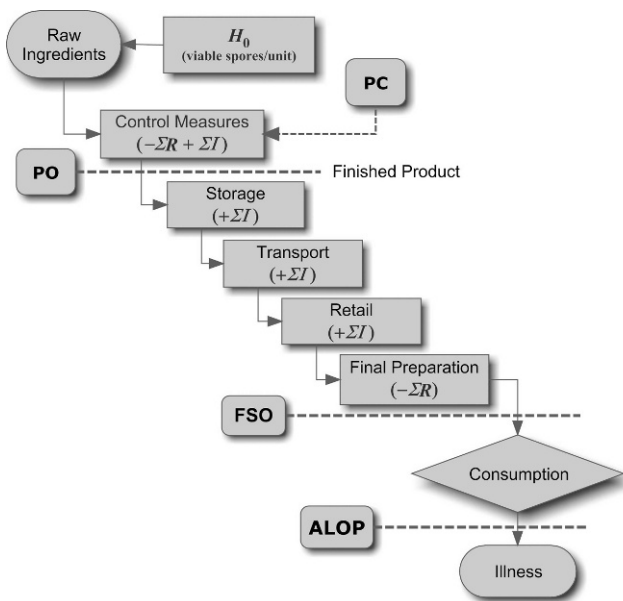
FIGURE 1. *Risk management framework for commercially sterile foods.*

processing to home canning and an emphasis on strictly following directions have generally been successful, but unfortunately errors continue to occur. Fortunately, the low number of units generally produced by home canning has limited the number of cases in outbreaks.

Although the toxin produced by *C. botulinum* is heat labile and most foods in the category considered are heated by the consumer before consumption, some foods are not adequately heated before consumption to inactivate the toxin, and therefore consumer heating cannot be considered as a suitable control measure. Because of the severity of the illness, the permissible level of toxin would necessarily be extremely restrictive. In practice, there is no acceptable level of *C. botulinum* toxin in food. Although toxin production is associated with a proliferation of *C. botulinum,* toxin production cannot be controlled or limited and is therefore not considered a useful basis for management.

RISK MANAGEMENT FRAMEWORK

General principles of the risk management framework. Particular guidelines on microbiological risk management were developed by the Codex Committee on Food Hygiene *(23)*. These guidelines introduce a risk management framework that supports public health protection and facilitates international trade. The risk management framework illustrated in Figure 1 builds on earlier work of the ICMSF *(49)* and incorporates a number of risk management concepts that are briefly introduced below.

Scope. Choosing the risk level that is deemed an appropriate or tolerable PHG is the responsibility of a competent authority and must be consistent with regulatory policies and public opinion. In the context of international trade, the expression of risk in the population is the ALOP. The ALOP is used to describe the acceptable level of risk

established by an importing country that should not be compromised by the exporting country's product *(115)*. The ALOP is converted to an FSO, which in turn can be used to set POs, a suitable control measure used by the food industry. Establishment of the ALOP and setting the FSO for commercially sterile foods are discussed below.

PHGs and the ALOP for commercially sterile foods. Defining the risk to the consumer is based on deciding what level of risk (ALOP or PHG) will be tolerated in the food product. Ideally, this decision is based on scientific evidence of the magnitude of concurrent risk (e.g., epidemiological data or historical evidence) and consideration of whether the proposed level of risk is achievable in practice. Ultimately, the decision is one of public policy. For a country, a competent authority can stipulate a PHG. The goal can be related to insight on the concurrent number of illness cases caused by a certain pathogen and a decision on whether that level needs to be reduced and to what extent. Risk can be expressed as a number of human cases per million per year associated with a foodborne pathogen. When the risk expression specifically refers to the relevant food, the risk can then be expressed as the likelihood of incurring a food-related illness from a serving of that food. Depending upon virulence characteristics of a pathogen, a PHG may be designated for specific populations that are more susceptible. Any deficiency in a process designed for a low-acid, shelf-stable food may be a major public health concern because of the severity of botulism and the widespread distribution of these products *(18)*.

Reliable data on the annual volumes of low-acid canned food product categories manufactured over a given number of years with recorded cases of illness attributed to them would allow an estimate of the proportion of cans causing illness per number of cans marketed. Unfortunately, too few data are suitable to allow a quantitative safety assessment. However, after research was done to determine what heat treatment was required to kill spores, the canning industry and government regulators went to great lengths to make sure that industry processed food sufficiently to ensure that every can was safe *(15)*. Industrial practices used to deliver a safe canned food product are currently based on the concept of commercial sterility, where the food product is processed such that under normal storage conditions no microorganisms will grow—either those that pose a public health hazard or those that affect only quality. Based on scientific evidence, over the lifetime of the canning industry relatively few cases of *C. botulinum* intoxication have been attributed to industrially produced, commercially sterile, low-acid canned foods.

The Centers for Disease Control and Prevention *(17)* reported that before the July 2007 botulism outbreak associated with one firm's hot dog chili sauce, only four of the outbreaks associated with commercially processed foods (i.e., canned tuna, liver paste, vichyssoise, and beef stew) were linked to deficiencies in a commercial canning process. The last such outbreak in the United States occurred in 1974 and was associated with commercially canned beef stew.

Some products, such as shelf-stable canned cured meats, have never been implicated in a botulism outbreak *(100)*. Peck and Stringer *(71)* reviewed many of the recent outbreaks of foodborne botulism, and although outbreaks have been associated with commercially canned foods from many countries, none of these outbreaks have been associated with a properly processed product. Because no known cases of botulism can be attributed to properly processed commercially sterile foods, it is difficult to establish an accurate production-based level of risk. The problem with a production-based approach to managing risk is that it leads to the assumption that a country with a large production volume would need a more stringent process than a country with a smaller production volume or would be willing to accept a greater number of cases of illness. Similarly, small producers making small numbers of units per year might incorrectly believe that their processes can be less stringent than larger producers who produce millions of units per year. In fact, regardless of quantity produced, the aim is to achieve the same level of protection with the same target endpoint.

Considering the severity and life-threatening nature of botulism, the relatively high mortality rate associated with ingestion of *C. botulinum* toxin, and the susceptibility of humans to the toxin, any incidence of botulism is considered unacceptable. Thus, when taking into account uncontrollable risk, the ALOP approaches zero cases in any production.

Any alternative to existing thermal processing technologies, such as high pressure processing, will likely be required to deliver an equivalent level of protection. For two processes to have an equivalent level of protection, each process would need to result in an equal probability that a single unit of commercially sterile food would not contain a viable *C. botulinum* spore. However, with a target ALOP of zero cases of botulism for every production batch, it is very difficult to determine the safety level of any new process employed without having products consumed for decades after implementation.

Setting an FSO. An FSO is proposed as an intermediate public health target in the risk management framework *(25)*. The role of an FSO is to help translate a PHG or ALOP into more meaningful targets for the industry involved in producing and marketing the food concerned. These targets are outcome oriented, which means that they provide explicit guidance on the level of a hazard tolerated at a particular point in a food chain without specifying by which measures the hazard level is controlled. This approach provides flexibility to the industry when establishing suitable food safety management systems using the control measures of their choice and within their capabilities.

The presence of *C. botulinum* toxin produced by surviving spores is a hazard for low-acid, shelf-stable foods. Given the extreme toxicity of this toxin and the inability to control or limit production of toxin in a low-acid food stored for extended periods at ambient temperature, the FSO would be framed as the maximum frequency of occurrence of one or more viable spores capable of growth in the food. As proposed by the Codex *(26)*, an FSO may be set only by

competent authorities. In deriving an FSO from an ALOP or PHG, the competent authority must determine how the food being produced contributes to the number of cases of foodborne illness caused by the pathogen of concern.

A traditional risk analysis approach might use total production as a basis for formulating a target FSO. For example, the U.S. production of low-acid canned foods is on the order of 10^{10} packages (cans, pouches, or jars) per year. During storage, shelf-stable foods have sufficient time and are exposed to temperatures favorable for bacterial growth and toxin production; therefore, a reasonable assumption for foods that support the germination and growth of *C. botulinum* is that one surviving spore in a package could result in toxin formation. Thus, the FSO could be expressed as no surviving spores of *C. botulinum* capable of outgrowth in 10^{10} packages of food that can support toxin production.

An alternative view is that each processor is statistically independent, which seems logical because each processor encounters different levels and types of variability. Variability exists in the source of raw materials, the bioburden of spores associated with those materials, the processing method, and the formulation. If statistical independence is assumed, an individual producer need only ensure that a given food batch does not contribute significantly to the risk of an incidence of botulism. Regardless, the use of total production or independent batches makes an appropriate FSO impossible to calculate because no outbreak has been attributed to a properly processed product.

Current practices that employ reduction and inhibition measures, either alone or in combination, have resulted in meeting the desired ALOP of zero cases of botulism in any population; therefore, the target treatment currently being used is an appropriate starting point for setting an FSO. For example, Hauschild and Simonsen *(46)* estimated that safe shelf-stable canned cured meats are produced with $<10^{-7}$ to 10^{-8} probability of spore outgrowth per unit. Pflug *(73)* recommended a 10^{-9} per unit target for the probability of outgrowth of *C. botulinum* in shelf-stable foods that support growth of *C. botulinum* and toxin production. Pflug's *(73, 75)* underlying rationale for heat processing of low-acid canned foods is based on conservative estimates that the initial bioburden of the target microorganism present in the food is approximately 10^3 *C. botulinum* spores per unit. Thus, for a 12-log inactivation, a reasonable FSO would be approximately -9.0, which can be expressed as the probability of no spores capable of growth in 10^9 units of a food that supports *C. botulinum* germination, growth, and toxin production. Decades of commercial experience with the criteria of commercial sterility supports the implied ALOP and FSO as being achievable and effective. However, any change in current practices would necessitate a reevaluation of the FSO.

When designing a process, the processor will want to take into consideration both the log reduction of *C. botulinum* spores and the conditions within the food matrix that contribute to the inhibition of spore growth and toxin production. The proposed FSO is applicable as a management target only for foods that support *C. botulinum* growth

and toxin production. When the food safety management system put in place by the industry can assure that there is no possibility of growth (e.g., high-acid canned foods or low-acid canned foods with suitable preservative factors), an FSO based on an absence of spores is not the most appropriate criterion. The metric for hazard control (still based on prevention of toxin formation) is assurance of no growth.

When considering an incidence of human botulism, survival of a viable spore is not the only factor that contributes to the hazard. The consumer also must purchase and consume the product. For example, the spore must survive the treatment, germinate, grow, and produce toxin without swelling the container, the resulting putrefaction must not be objectionable to the olfactory senses of the consumer, and the product must be consumed. As a result, additional protection may be gained from the influence of these factors. In fact, estimates of safety would tend to be somewhat higher than the true *C. botulinum* hazard level *(46)*. Pflug *(74)* estimated that when the probability that a viable spore survives is 10^{-9}, the *C. botulinum* hazard level is likely on the order of 10^{-11} to 10^{-13} because of consumer handling. Considering the variability inherent in this added potential safety from consumer handling, it is not recommended that this factor be added to the level of safety delivered by the process when determining whether the process meets or exceeds the FSO limit.

When validating a proposed food process to determine whether an FSO can be achieved, certain assumptions and inferences are required. The risk manager should consider the validity of inactivation data extrapolated from high spore concentrations to probabilities that are based on a low spore number in millions of cans. Additional points of validity include pilot plant and laboratory data that are used to characterize the performance of a commercial-scale process and the absence of injured spores that might have lengthy germination times.

Basis for an FSO. When establishing a typical thermal process, care must be taken to ensure that the most slowly heating container receives adequate treatment; therefore, the level of risk is characteristic of that associated with a unit, or one container. In this document, the FSO is expressed on a per unit basis because regardless of lot size or endpoint specification, the analysis was conducted using the same unit of measure. At times, the risk manager might consider a more conservative FSO because one package often has more than one serving and more than one serving may be consumed within several days.

CONTROLLING INITIAL LEVELS, H_0

When using a risk-based approach to process development, it is necessary to quantify the initial bioburden of the raw materials to implement appropriate control measures and thus meet the target FSO. Ideally, quantitative data (count or most probable number [MPN]) from a sufficient number of samples in a study with a significantly large sample size would be used to assess the distribution of organisms. Spores of *C. botulinum* are widely distributed in the environment and are present in a wide variety of foods; however, development of the best quantitative estimate of H_0 can be difficult because the prevalence of microbial pathogens (e.g., *C. botulinum*) in food ingredients is generally low and difficult to quantify.

Scope. Because of the breadth of *C. botulinum* research conducted, only the most relevant scientific information constituting the basis for the estimation of the components of the H_0 term are mentioned here.

Objective. The objective of this section is to provide guidance for the estimation of the value of the H_0 term in the FSO formula. A set of tools is provided to support this task, and the technical language required for efficient communication of related concepts is presented.

Expressing H_0. H_0 is the initial level of the hazard *(49)* and can be expressed using equation 2:

$$H_0 = \log(N_0) + \varepsilon \qquad (2)$$

where H_0 is defined as the log of the number of spores present per unit of the product (N_0) before implementation of any control measure, and ε is unknown error. H_0 should be described in quantitative terms. For greatest value in decision making and setting performance criteria, H_0 should be described as a distribution to reveal whether it is normal, lognormal, or some other form.

Estimating H_0. Skinner *(82)* compiled an extensive database of published articles on *C. botulinum* that can be accessed through the IFSH Web site. Given the breadth of *C. botulinum* research conducted, this database can serve as an excellent starting point for a literature search on the incidence of *C. botulinum* in numerous products and raw materials. A number of articles referenced in the database contain distribution data on *C. botulinum*. Other possible sources of information on the incidence of *C. botulinum* include unpublished survey data from industry, government agencies, universities, and trade organizations.

The literature should be surveyed for data available on the prevalence and incidence of *C. botulinum* in various raw foods and ingredients. Several questions should be considered when evaluating earlier survey data for inclusion in an analysis:

- Were the numbers and sizes of samples analyzed representative of the ingredients or product of interest?
- Was the number of samples adequate to evaluate normal variation in the ingredients or product?
- Where in the food chain were the samples collected?
- Was the methodology used adequate to accurately detect and enumerate *C. botulinum* spore numbers in the sample?
- Does the study represent the region of the world of interest?
- Because methodology has continually evolved, would the published surveys yield different results for prevalence and incidence of *C. botulinum* if conducted with current methods?

The source of the data and the method used for their collection should be documented. The confidence in the quality of the data and applicability to the situation of interest should be recorded. The quality of the estimate increases as the amount of available ''good'' data increases; however, when fewer data are available, a broader distribution still may provide a useful estimate of the H_0. Regardless of the value selected for the H_0, any estimates must err on the conservative side by overestimating the bioburden of incoming raw materials.

Although some distribution data exist for certain food groups, most data generated are not quantitative. Studies typically have involved placing the sample into an aliquot of medium, typically Trypticase–peptone–glucose–yeast extract broth or cooked meat medium, and incubating at either 35 or 25°C for proteolytic and nonproteolytic organisms, respectively. After incubation, a small sample of the broth is usually tested by using the mouse bioassay method, in which the aliquot of liquid sample is centrifuged and injected intraperitoneally into mice. Mice that die with symptoms characteristic of botulism are considered positive for the presence of C. botulinum toxin, indicating C. botulinum organisms were present in the original sample. Some studies have involved determination of the specific type of C. botulinum spore present by identifying the type of toxin produced: A, B, E, or F. Other studies in the literature have gone even farther by performing MPN studies, which actually provide an estimate of the number of spores in a sample. Enumeration using MPN methods is expensive and time-consuming and therefore has not been done in the majority of studies. Although most C. botulinum distribution data in the literature are given as spores either present or absent in a measured mass of food or soil, presence-absence data for the same commodity may be used to generate MPN estimates. Halvorson and Ziegler (41) introduced a means of translating presence-absence data into a quantitative estimate with a standard deviation. Accuracy of this method is dependent on both the number of tubes and the sample size and bacterial population.

Survey data for C. botulinum frequently will include many ''nondetectable'' values and a few high counts. A histogram of such data will exhibit a long tail. In practice, such data are often best approximated with a lognormal distribution; taking the logarithms of individual counts will make the data approximate a normal distribution. Because ''nondetectable'' values may be obtained, a positive constant should be added to all counts prior to taking the logarithms. If a mathematical distribution can be fitted to a given set of data, the upper limit, perhaps at the 95% or 99% confidence level, can be chosen. This limit should not be exceeded if the raw materials continue to exhibit the levels obtained during the initial survey. However, bioburden data for a given product may not precisely follow a well-recognized distribution. In this situation, control charting, which has successfully been applied to medical device and radiation sterilization (4), is an appropriate method for setting limits. Control charts do not strictly depend on any underlying assumed distribution. Rather, the quantities plotted on control charts are the mean and standard deviation (or range). Control limits for a standard Shewhart control chart of the average bioburden can be established after a sufficient number of historical counts have been collected (4).

What do we do in the absence of data? In the absence of data, one option is to conduct surveys to determine prevalence and numbers; however, the cost of conducting significantly large surveys for C. botulinum, including housing and processing mice, and strict regulations governing the possession, use, and transfer of select agents (42 CFR 73 (111) and similar international regulations) are factors that may limit collection of significant amounts of data regarding distribution of C. botulinum spores. However, new laboratory methods are being developed to make screening and quantification of C. botulinum spores easier. PCR methods are being utilized to identify and type C. botulinum obtained from enrichment and growth cultures of C. botulinum spores from food or soil samples, which would allow screening of samples before enumeration. However, PCR methods have not yet been established as sufficiently robust or quantitative. Enzyme-linked immunosorbent assays may be used for quantitative estimates of C. botulinum toxin and to identify toxin type. Methods such as immunoseparation are being investigated for their potential to concentrate C. botulinum spores from a sample, which would allow the evaluation of larger samples and thus easier detection. In addition to the physical methods for detection, significant work is being done on sampling plans (112) to minimize the number of samples necessary to obtain useful data. The Chilled Food Association's sustainable shelf life extension LINK research program is designed to provide more accurate spore concentration data through a better understanding of sampling plans (3).

Caution should be exercised when new surveys are conducted because the resulting data may not be truly representative for all situations because of factors such as seasonal variation and geographic differences. A single survey may result in inadequate and misleading estimates of bioburden. Multiple surveys may be required for the resulting data to be truly representative for all conditions that may influence the prevalence of the organism.

When a quantitative estimate becomes impractical or perhaps even impossible to obtain, other options should be considered.

Suggested worst-case estimate for H_0. Factors known and unknown can influence the estimate of H_0. Thus, it may be desirable to increase the estimate to allow for contingencies and a worst-case scenario. Because an estimate for certain pathogens may be grossly inaccurate and generating new data may be prohibitively expensive, addition of some safety factor to generate a conservative estimate may be the only recourse. Although a worst-case estimate is meant to give confidence in its conservatism, one must be mindful of what is realistic. Caution is needed in defining a process using the worst case, taking into consideration process dependent variables. Generally, root

crops (e.g., onions, garlic, beets, carrots, and potatoes), mushrooms, some spices, and honey are likely to have relatively high spore loads. A survey of existing data may give an indication of the worst case.

Surveys of soil samples should be reviewed, particularly if the data could yield any information on the incidence and prevalence of *C. botulinum* on specific commodities grown in these soils. When only soil survey data are available, one could extrapolate a worst-case estimate for many raw ingredients such as onion, carrot, garlic, or even seafood.

Because *C. botulinum* is only one of the broad group of microorganisms known to have the characteristics of a mesophilic putrefactive anaerobe (PA) and spore former, data on a similar microorganism or group of microorganisms in the ingredients or food could be used to develop an estimate for the presence or level of *C. botulinum* in this food. In certain cases, current restrictions for handling certain pathogens make it much less likely that an industrial laboratory will conduct a survey for a biological safety level 2 pathogen (e.g., *C. botulinum*) in ingredients and foods. Thus, industrial laboratories must rely on testing for a broader group of microorganisms that is more likely to consist of a nonpathogenic PA and must remain conservative with their estimates.

Riemann *(79)* reported up to 51 anaerobic spores per g in individual samples, and one pork luncheon meat sample contained 15 clostridial spores per g. Hauschild and Simonsen *(46)* reported a ratio of 10,000:1 for PAs to *C. botulinum* spores. Greenberg et al. *(40)* found only one *C. botulinum* strain among 19,727 PA isolates from 2,358 samples of raw meat and poultry, whereas the majority of samples yielded PA spores. Hence, the incidence and/or level of PA spores seems to be somewhere between about 2,000 and about 20,000 times greater than the incidence and/or level of mesophilic *C. botulinum* spores, based on limited data. Therefore, consideration must be given to the scientific basis for accepting such data as reasonable estimates of H_0. The acceptability of alternative microorganisms and methodologies should be agreed upon both by industry and the appropriate regulatory agency before surveys of ingredients and foods are conducted.

The derivation and any increase to protect against worst-case situations in the development of the estimate of H_0 must be done in an open and transparent manner. The process should be documented so others may consider agreement or, if not, provide a rationale for a different value.

Changes in ingredients, formulations, and manufacturing. Product formulations used in commercial practice today differ significantly from those that were used more than 20 years ago. Many manufacturing facilities have been changed or newly built to increase processing efficiency and the quantity and type of products produced. The changes generally have brought improvements in cleanability of equipment and microbial control when these factors have been a concern.

Gaps in the literature for H_0. Most distribution data for *C. botulinum* were obtained before the 1980s in response to an outbreak from a particular food or food type or some targeted food area of concern. Therefore, information from a variety of sources may be helpful for estimating H_0. For example, epidemiological data may indicate that certain ingredients or products are of higher risk because they have been implicated as the source of foodborne illness. Data also may indicate that the pathogen has been implicated in diseases associated with wild and domestic animals.

Abundant data exist for *C. botulinum* in soil collected from different regions of the world and in aquatic environments. Such data could suggest the likely presence of the pathogen in an ingredient (e.g., onions or fish) harvested from certain regions. The 1963 outbreak of botulism in the United States was attributed to type E *C. botulinum* toxin formation in temperature abused smoked whitefish chubs from the Great Lakes region. This outbreak included cases in Tennessee, Alabama, Kentucky, and Michigan and was large enough to prompt food microbiologists to initiate studies of the distribution of *C. botulinum* spores in fish, fishery products, and their environments. Numerous surveys were conducted around the Great Lakes area and included fish, water, and sediment. This inquiry also expanded to the Pacific Coast and others areas in the world where information was collected on the distribution of *C. botulinum,* particularly proteolytic type E and nonproteolytic B isolates, in fishery products and sediment. These investigations garnered a large amount of data for *C. botulinum* spores in fish, fishery products, and sediment worldwide.

Outbreaks of infant botulism stimulated research related to the presence of *C. botulinum* spores in honey and infant foods. Questions related to the safety of surimi-type products (refined fish protein products) and the distribution of *C. botulinum* spores in these products led to the generation of data. Surveys of various lunchmeat products also have been performed. Other than certain targeted food areas, however, these data are limited.

Recommended research required to fill gaps. Research could be undertaken to identify any correlation between numbers and distribution of *C. botulinum* and other bacteria. To generate data for a new processing technology, a company may need to gather data for a particular food or group of foods. Estimates can be based on conservative numbers generated in such a study. When evaluating only a small number of samples, care must be taken to determine whether these sample are representative because numbers may change within country of origin or region. Ideally, the available data will represent the level of the pathogen in the food just before the reduction step. Because of the unique formulation of most commercially processed foods, such a study should be conducted at the plant to establish contaminant levels and variability over multiple lots of production. For raw materials that are commonly used by multiple food processing firms in the production of value-added products, it may be possible to conduct an industry survey in which a number of stakeholders such as

academics, contract laboratories, and government agencies jointly participate in generating survey data.

TOTAL REDUCTION OF HAZARD, ΣR

Few, if any, life forms are more resistant to chemical, physical, and biological agents than bacterial spores. However, the food industry has been able to develop processes that enhance or diminish the resistance properties of spores, either by manipulating intrinsic genomic and biophysical properties or through treatments such as heat and pressure. Understanding the ability of a process to deliver the necessary lethal dose (transport phenomenon) and the behavior of spores within the food product are important factors in determining whether a process has rendered the microbial contaminants inactive.

Reference is often made to the combined effect of exposure to lethal or sublethal agents and the impact of pathogen spore recovery (the ability to repair and exhibit viability) when describing the delivered process treatment. However, to implement the FSO approach appropriately new tools are needed to quantitatively separate these two effects. The effect of the exposure to lethal or sublethal treatments is incorporated into the measurement of ΣR. The ability of a spore to repair and exhibit viability, as affected by the inhibitory properties of the food product, is incorporated into the measurement of ΣI.

Spore resistance is generally determined by measuring the ability of a spore exposed to a lethal or sublethal agent to survive, germinate, and outgrow to become a vegetative cell. Once the primary cell emerges from the spore, it can further subdivide to the point at which the bacterial population that develops can be counted. Any interruption along the continuum from initiation of spore germination through emergence of the primary vegetative cell and subsequent cell division will be assessed as nonviability and will enter into corresponding calculations of spore resistance. In contrast, the ability of the food product to prevent the initiation of germination and outgrowth is a function of ΣI. Therefore, spore resistance must be considered as the combination of biological activities occurring both during and after processing.

Scope. The main concepts and procedures needed for the appropriate estimation of the term that describes inactivation or removal (ΣR) of the health hazard are presented. Sterilization processes of practical importance to the food industry, including heat, radiation, and chemical sterilization, and some emerging technologies such as high pressure sterilization are discussed. This discussion is restricted to concerns with public health and does not explicitly address economic spoilage concerns and process deviations; however, the concepts and procedures presented are applicable to inactivation of microorganisms even when microbiological contamination may not have an impact on the consumer safety of the product.

Several reviews of the literature have thoroughly discussed the various reduction-processing technologies; therefore, only the most relevant scientific information

constituting the basis for the estimation of the components of the ΣR term are mentioned here.

Objective. The objective of this section is to support the estimation of the value of the ΣR term in the FSO formula. A set of tools is provided to support this task, and the technical language required for efficient communication of related concepts is presented.

Expressing ΣR. ΣR is the cumulative reduction of the hazard (49). This dimensionless number is defined as the log ratio of N_0' to N_R as given by equation 3:

$$\Sigma R = \log\left(\frac{N_0'}{N_R}\right) + \varepsilon \qquad (3)$$

where N_0' is the number of spores per unit that are present at the implementation of the control measure and are capable of growth under ideal growth conditions, N_R is the number of spores per unit that are present after the reduction step(s) and are capable of growth in the product, and ε is the unknown error.

Control measures. One method for describing a reduction process is to view the collective function as a control measure. A control measure is any unit operation that results in control or reduction of the target organism, including the actual transport phenomenon related to treating the food product and attenuators of resistance, the physical or chemical characteristics of the food, and the treatment effects that augment the dynamics of the process. Figure 2 is an integral representation of how the elements of a control measure manifest themselves as the reduction kinetics that are ultimately measured and/or modeled for the process. Within each control measure there will be control points and/or critical control points that must be regulated and monitored for the control measure to be effective.

Processing techniques that can be used for the reduction of microbial loads include removal and inactivation. Removal unit operations include filtration, centrifugation, and washing, which belong to the group of hydrodynamic processes that are driven by a hydrostatic or hydrodynamic pressure gradient. Inactivation unit operations include a number of physical and chemical agents capable of pronounced bactericidal and/or sporicidal effects. A summary of different unit operations that have been used within the food industry to deliver a lethal treatment is presented in Table 3.

The effectiveness of a unit operation is dependent on both the measured transport phenomena and how the microbial load responds to the treatment. The ability of spores to survive, germinate, and grow within a food product is dependent on the resistance of the microorganism and of the constitutive properties of the food. The constitutive properties of a food product may augment the unit operation and impact the growth and survival of the microorganism.

Attenuators weaken or reduce spore resistance, and augmenters increase resistance. Table 4 defines the generally recognized determinants of spore heat resistance. Many of the determinants may play either an attenuator or an augmenter role depending on the circumstances of use.
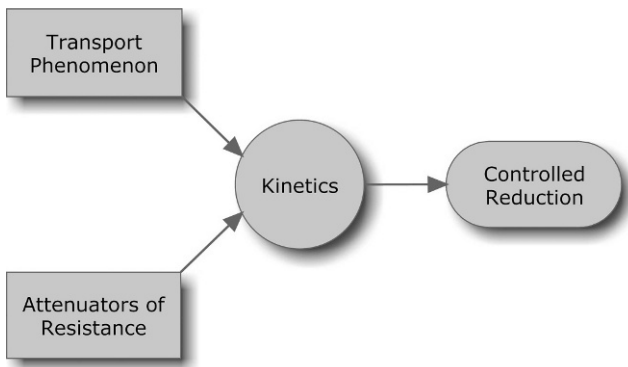
FIGURE 2. *Components of a control measure system resulting in a reduction of microbial contaminants as described by the kinetics of the unit operation.*

One of the most studied product parameters that impact spore heat resistance is pH. As early as 1919, Dickson et al. *(28)* reported the impact of pH on spore heat resistance. Xezones and Hutchings *(116)* determined the impact of unprocessed food products of pH 4.0 to 7.0 on spore heat resistance of *C. botulinum*. Spore *D*-values were 30 to 50% lower in pH 5.0 product than in pH 7.0 product. In that study, spores were heated in product and recovered on agar. Other researchers have similarly demonstrated the impact of pH on spore heat resistance *(47, 57, 80)*. The measured heat resistance found by Xezones and Hutchings *(116)* might have been even lower had the researchers enumerated survivors in product rather than in the neutral-pH pork infusion agar.

Water activity (a_w) also impacts spore heat resistance. In general, spore heat resistance is greatest at low a_w values (0.2 to 0.4) and lowest as the a_w approaches 1.0 *(63)*. Several studies have revealed the impact of salt on spore heat resistance. Salt added to a heating medium generally increases resistance to a point. The deleterious effect of NaCl most likely impacts the recovery of heat-damaged spores. However, salt likely plays a minimal attenuating role during heating because NaCl will lower the water activity and potentially increase heat resistance. When NaCl

TABLE 4. *Determinants of spore heat resistance*

Determinant
Strain and species under investigation
Temperature and other sporulation conditions
Nutritional content of food product
Acidulant and pH of food product
a_w of food product
Temperature of product storage
Fat and/or oil content
Solute concentration in food product (e.g., NaCl, NaNO$_2$, KCl, CaCl$_2$, sugar)

is added to the recovery medium, heat-damaged spores recover to a lesser extent as the NaCl concentration increases. Hutton et al. *(47)* found a 20 to 30% increase in measured spore heat resistance when up to 2% salt was added to the recovery medium. Although these researchers did not quantify the effect of NaCl during heating, they speculated that salt impaired the ability of heat-damaged spores to grow and form colonies on agar plates.

Other preservatives such as nitrite also impact spore heat resistance, most likely inhibiting the recovery of heat-damaged spores.

Mathematics of inactivation. Microorganism inactivation that is dominated by the transformation of a single population has been well described by a pseudo first-order mathematical model, Chick's model *(20)*. For thermal sterilization, it is

$$\frac{dN}{dt} = -kN \tag{4}$$

where N is the number of survivors, k is the rate constant, and t is time.

If the effect of concentration of the lethal agent must be taken into consideration, the Chick-Watson model can be used:

$$\frac{dN}{dt} = -kC^nN \tag{5}$$

TABLE 3. *Example unit operations used to reduce microbial loads*

Technique	Process(s)	Mechanism(s)	Driving force	Other bactericidal mode
Physical removal	Filtration, centrifugation, and cleaning	Momentum transfer	Hydrostatic or hydrodynamic pressure	
Thermal inactivation	Direct and indirect heating	Heat transfer	Temperature	
	Ohmic, microwave, and radio frequencies	Electromagnetic energy	Temperature	
	High pressure	Momentum transfer and heat generation	Differential pressure	Temperature
	Ultrasonication	Momentum and heat transfer	Pressure	Temperature and cavitation
Nonthermal inactivation	Irradiation	Electromagnetic energy	Photon and electron transfer	Ionization
	Gamma, X-ray, and UV irradiation			

where C is the concentration of the lethal agent and n represents the effect of changes in the concentration in the reaction rate. For instance, for $n = 1$ (ethylene oxide), when the concentration doubles, the rate also doubles, whereas for $n = 3$ (some phenolic compounds), when the concentration doubles, the reaction rate increases by a factor of 8.

In the field of sterilization science and technology, the rate constant has been historically replaced by the decimal reduction time (D-value), which is the time it takes the decimal logarithm of the survivors versus the time to go through an order of magnitude (or a log cycle). For thermal sterilization, the D-value is inversely proportional to the rate constant. In the case of the Chick-Watson model, the D-value is inversely proportional to the rate constant multiplied by the concentration to the nth power.

For constant lethal conditions (e.g., temperature, concentration, and pressure), as long as the system consists of a single transformation (i.e., inactivation) and a single population (i.e., typically dormant, clean, and clump-free genetically pure bacterial spores), the graphical representation of the logarithm of the survivors versus time will often approximate a straight line. The intercept of the curve corresponds to the initial number of survivors (N_0), and the negative reciprocal of its slope corresponds to the D-value for the set of conditions used in the test. Experimental and biological variability induce deviations from a perfect straight line. Systematic or excessive deviations (a process authority should define these when the conditions are deemed significant) must be investigated before the model can be applied to practical problems. The focus should be on the application of the simplest model possible that can adequately describe the process.

The transient case, in which temperature is a function of time, requires that the function that describes the effect of temperature on the rate constant (D) is known or developed. For that purpose, the rate constant is appropriately described by the activation energy in the Arrhenius equation, or the parameter z, which is the number of degrees that it takes the log (D) versus temperature curve to go through one order of magnitude. Both models work reasonably well in the temperature ranges of practical importance. The Arrhenius model is a better descriptor for temperatures that are significantly different from the reference temperature.

Traditionally, equation 5 leads to formulas that can be used to evaluate the effectiveness of a thermal sterilization process. Similar formulas are available for chemical and high pressure sterilization.

Equation 6 is the equation for the estimation of survivors of a transient thermal sterilization cycle:

$$N(t) = \frac{N_0}{10^{\left[\frac{1}{D_{121.1}} \int_0^t 10^{\frac{T(t)-121.1\,°C}{z}} dt\right]}} \tag{6}$$

where $D_{121.1}$ is the D-value at 121.1°C, t is time, and T is temperature in degrees Celsius.

Equation 7 is the equation that estimates the accumulated lethality, or sterilization value, of a process. When we define the reference temperature as 121.1°C (250°F) and the z-value

as 10°C (18°F), the formula defines the F_o of a process:

$$F_o = \int_0^{time} 10^{\frac{T(t)-121.1\,°C}{10\,°C}} dt \tag{7}$$

The spore log reduction (SLR) of a process can be expressed as in equation 8:

$$\text{SLR} = \log\left(\frac{N_0}{N_R}\right) = \int_0^{time} \frac{dt}{D(T(t),C(t),P(t),\text{pH},\ldots)} \tag{8}$$

where N_R is the number of survivors at the end of the reduction step, N_0 is the number of survivors when $t = 0$, and D is a function of the significant parameters of the system (e.g., temperature, pressure, and pH). This equation, coupled with the availability of mathematical software to perform the numerical integration, makes the calculation of the survivors of a lethal process straightforward regardless of the complexity of the function that describes D. Therefore, it becomes possible to estimate the number of survivors of lethal treatment combinations such as temperature, pressure, and concentration.

Other transformations may dominate some processes. For instance, sterilization of cured meats is driven by injury transformation. Injured spores of *C. botulinum* will not grow in the presence of NaCl and NaNO$_2$, and the rate of injury is significantly higher than the rate of inactivation. Therefore, the kinetics of spore injury will drive the process and successfully produce high pH shelf-stable products using a small fraction (as little as 0.6 min) of the F_o otherwise required.

Sample size. In general terms, triplicate studies under actual worst-case production conditions are required. These worst-case conditions will be determined by the process authority and may include set-point temperature (forced to the lower end of the allowable range), headspace, solids:liquid ratio, drained weight, starch (or other ingredients that affect the viscosity), and concentration. The selected set of conditions must force a situation in which the probability of this combination of factors actually occurring in a production run is very low.

The use of small sampling theory (Student's t distribution) provides flexibility because the prediction, tolerance, or confidence intervals will increase when the sample size decreases. A minimum of 10 replicate points likely will provide enough data to attain satisfactory descriptions. In some cases, sterilization processes may be validated using a destructive sterility test combined with a high challenge (i.e., 1 million spores per unit).

Surrogates and biological indicators. The characteristics of the surrogates or biologic indicators should closely follow the corresponding recommendations presented in the appropriate sections of the U.S. Pharmacopoeia USP30-N25 *(110)* or equivalent. Deviations from these requirements must be understood and justified by the process authority.

Variability. Commercial sterilization is conducted in large industrial equipment. Sterilizers are typically large chambers in which the lethal conditions are provided to the

product load under controlled conditions. The nature of industrial operations and of the product units results in variability in the lethality delivered to the product units in the load. Therefore, the sterilization goal should be the minimum required throughout the product load at the end of the controlled interval of exposure to the lethal agent.

The empirical approach is very powerful and useful under these circumstances because its main limitations typically are not significant. Industrial applications do not change often and are not intended as research tools. Empirical models provide little information about the process from the mechanistic viewpoint, and any change in the significant variables outside the region where experimental data were acquired during cycle development will most likely necessitate a revalidation of the process.

The appropriate use of empirical models is often the quickest way to define the set of parameters required to perform industrial sterilization processes successfully. Preliminary work through mathematical simulation and experimental exploration of the process dynamics and the experience accumulated dealing with similar situations often are the most efficient way to define the conditions to be used for the large-scale experiments related to cycle development and validation.

Once the set of conditions (e.g., temperatures, concentrations, and pressures) and their corresponding ranges have been defined and the equipment shown to be capable of delivering the intended cycle through successful installation qualification, operation qualification, and validation, one approach is to define experimentally the time of exposure to the controlled lethal agent in situ. Two other possibilities are determination of the time needed to accumulate a certain minimum F_o in the product load and determination of the time needed to reduce the potential microbiological contamination enough to achieve commercial sterilization conditions, as a minimum, in the load.

Variability of the processes implies that the problem will be associated with the probability of success in achieving the required goal and its associated confidence. Currently, the frequency distributions for the accumulated F_o and SLR have not been determined in general. Each case probably should be handled independently. When some mathematical frequency distributions or empirical histograms are available and validated, the number of standard deviations around the mean needed to achieve a certain level of risk can be determined accordingly. Otherwise, the Bienayme-Chevishev rule (or the Chevishev theorem) provides an absolute worst case independent of the frequency distribution that the data may follow. For instance, to be sure to include 90, 95, or 99% of the population, 3.16, 4.47, or 10 times the standard deviation around the mean will be required as a maximum.

This probability (90 to 99%) itself is often not good enough to reduce the risk of failure to a value that will lead to a successful commercial sterilization process. One strategy that may be used to improve on this situation is performance of the experiments under extreme worst-case conditions for the process parameters of importance, e.g., the lower extreme of the allowed set-point temperature range. The likelihood of a process occurring at these extreme values is typically extremely low in a well-controlled facility using modern equipment. Thus, the probability of worst-case conditions and the probability of failure combine to produce a level of risk that equates to commercially sterilized product. A process authority should perform this type of analysis.

Use of mechanistic models. Failure of reasonably designed inactivation processes that are calculated based on mechanistic mathematical models (rather than empirical data) will occur in extreme case conditions, not along the mean values. Therefore, process variability must be integrated in the estimation of the inactivation or of the time required to achieve the previously selected inactivation level. Often, the main sources of variation (for a steam sterilization process, for example) are the variations in temperature and of the parameters describing the kinetic behavior of the bacterial spores of interest. In both cases, conditions that represent realistic extreme challenges to the process must be selected, i.e., lower temperatures and larger D-values and initial contamination levels. Once these conditions have been selected, the corresponding calculations are straightforward using the formulas presented here for the survivors or the SLR. The examples provide a demonstration of methods that can be used to include variation within the process.

Use of empirical models. Empirical models offer the advantage of providing a representation very close to the actual practical application but have some limitations. Because a black box approach is used, empirical models are not recommended as research tools, and any potentially significant change in product, loading configuration, process, and other factors will require a full repetition of the development and validation work. However, industrial processes are not intended to change frequently, which partially mitigates the associated burden of process revalidation.

Of practical importance is the use of tolerance intervals to determine the process exposure time under actual production conditions required to accumulate a minimum F_o under worst-case conditions (e.g., cold spot or low process temperature) and the use of linear least squares to determine the exposure time under worst-case conditions required to achieve a determined reduction in the population of bacterial spores. Linear least squares have important advantages: linear combinations of mathematical functions can describe properly the inactivation curves of practical importance, and the parameters of the models have been demonstrated to follow the F distribution (no assumption needed). Knowledge of the frequency distribution enables the use of powerful statistical tools to generate prediction intervals and compare curves.

Gaps in the literature. The following list identifies gaps in the literature:

- Reliable data are needed concerning the effect of the different parameters of importance (pH, composition, attenuators, etc.) on spore resistance.

- The molecular mechanism of inactivation for moist heat, high pressure sterilization, and other inactivation processes must be determined.
- Simple models that can be used to design processes involving multiple inactivation mechanisms must be developed.
- Procedures used to acquire data must be standardized to assure that the data acquisition systems are properly calibrated (in particular, temperature) and that microbiological procedures prevent the generation of artifacts due to deficient techniques.
- Requirements for assigning a frequency distribution to a population when the Monte Carlo (MC) method is used must be defined. For instance, the use of software to try a large number of distributions to select the one that best fits the data should be carefully evaluated because the MC method depends strongly on the data quality and the accuracy of the frequency distribution. Survivorship and F_o data also are known for not following the available frequency distributions.

Recommended research required to fill gaps. The following list identifies recommended research to fill the gaps identified.

1. Evaluate the impact that various factors have on ''obtaining unquestionable survival curves'' *(19),* such as
   - genetic homogeneity ensured by isolating a single spore;
   - sporulation medium designed to produce spores possessing the highest degree of stability and resistance under conditions that emulate where spores are likely to sporulate;
   - precautions taken to set aside individuals that do not possess the characteristics of the majority of the population (e.g., density gradient centrifugation);
   - homogeneity of treatment conditions such as temperature, level of spores, concentration of disinfectant (if any), and water activity;
   - mated or occluded regions, which should be eliminated from the sample holders used; and
   - efficient neutralization of the sporicidal substance (if any) without effect on the recovery of the test organism.
2. Obtain reliable resistance data for *C. botulinum* spores to properly describe inactivation. The main factors of importance are pH, water activity, temperature, salt concentration, and nitrite concentration. The ability to generate meaningful resistance data for *C. botulinum* spores (strains of industrial importance) is the main obstacle for the efficient calculation of ΣR. Most organizations do not have the proper facilities required to work with *C. botulinum,* and even fewer organizations routinely perform thermal death studies.
3. Develop appropriate descriptions of moisture transfer in the steam sterilization range.
4. Continue work using mutant strains and genomics to explore the nature of spore inactivation due to different agents.
5. Use Fourier transform infrared spectroscopy and fourth derivative UV spectroscopy to explore the structures that may be related to spore inactivation under high pressure and moist heat sterilization.

TOTAL INCREASE OF HAZARD, ΣI

Because *C. botulinum* is commonly present in the environment, low levels of *C. botulinum* will typically be present in many foods harvested from soil, water, and other environments. From a public health perspective, the presence of even one viable spore must be assumed to result in outgrowth and toxin formation in a neutral pH food packaged in a limited oxygen environment. Because consumption of *C. botulinum* neurotoxin is so dangerous, food preservation techniques are designed either to destroy spores or to inhibit outgrowth of viable spores. Although the likelihood of multiplication of *C. botulinum* is quite low when the food is handled properly during all stages of processing, distribution, retailing, and consumption, loss of process control or inappropriate treatment of the food at any stage could result in *C. botulinum* spores surviving processing, outgrowing, and subsequently producing neurotoxin.

Attenuating factors most often utilized in the food industry for limiting outgrowth of *C. botulinum* spores are pH, water activity, redox potential, added preservatives, competing microflora, and temperature. The effectiveness of any single control measure is dependent on the complex nature of the food matrix, and in many instances hurdle technology is employed to ensure safety *(39, 52, 55)*. Commercial sterility also can be achieved and maintained through formulation of the product so that it does not support growth of spore-forming pathogens and through pasteurization to destroy vegetative pathogens. A hermetically sealed container is intended to maintain the commercial sterility of its contents and must be designed to be secure against the entry of microorganisms during and after processing. However, the anaerobic environment and a lack of competitive microflora inside a hermetically sealed container provide ideal conditions for an increase in levels of *C. botulinum*. Therefore, the use of a hermetically sealed container is as important to commercial sterility as formulation and processing.

Scope. The main concepts and procedures needed for the appropriate estimation of the term that describes the increase of the health hazard (ΣI) are presented here. Inhibitory formulations and hurdle technologies of practical importance to the food industry are discussed. Many scientific publications are available that address various methods for controlling and predicting outgrowth and toxin formation; therefore, only the most relevant scientific information constituting the basis for the estimation of the components of the ΣI term is mentioned.

Objective. The objective of this section is to explain how to estimate the ΣI term in the FSO formula. A set of tools is provided to support this task, and the technical language required for efficient communication of related concepts is presented.
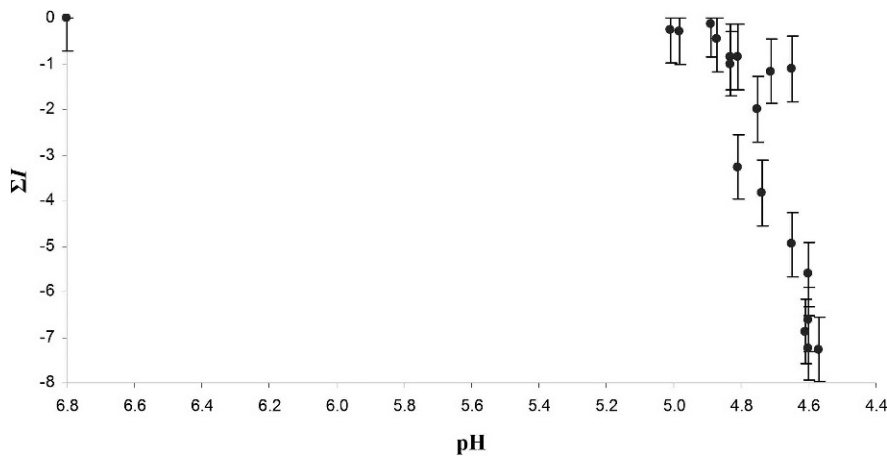
FIGURE 3. *Effect of pH on the growth of a single vegetative cell of* C. botulinum *type A strain ZK3 in peptone-yeast-glucose-starch medium incubated at 30°C for 14 days. Diamonds represent experimental results; bars represent 95% confidence intervals (60).*

Expressing ΣI. ΣI is the cumulative increase of the hazard *(49)*. This dimensionless number is defined as the log ratio of N_I to N_0' as given by equation 9:

$$\Sigma I = \log\left(\frac{N_I}{N_0'}\right) + \varepsilon \qquad (9)$$

where N_I is the number of spores per unit capable of growth in the product, N_0' is the number of spores per unit present at the implementation of the control measure capable of growth under ideal growth conditions, and ε is unknown error.

Control measures. A brief description of each control measure and the limits for controlling outgrowth of *C. botulinum* endospores are provided below.

Impact of pH on growth of *C. botulinum*. Increasing the acidity of a food product alone or in combination with other technologies can be effective for rendering the food safe and shelf stable. A pH of <4.6 has long been accepted as the limit for germination and growth of spores of *C. botulinum* group I (proteolytic) strains. This limit includes a 0.2 pH margin of safety based on the lowest pH (4.77) in which *C. botulinum* grew in a food at the time the limit was established *(43)*. A pH limit of 5.0 is widely accepted for group II (nonproteolytic) strains *(93)*. Because of the boundary effect exhibited by *C. botulinum* under acid conditions, shelf-stable foods of pH <4.6 are not required to be processed to inactivate *C. botulinum* spores. However, the buffering effect of the food must be considered when relying on pH alone for product safety and stability. Growth below pH 4.6 is possible in the presence of other organisms, namely *Bacillus* spp. *(77, 83)*. Growth also can occur at pH 4.2 in the presence of *Aspergillus gracilis* under tightly controlled laboratory and otherwise optimal growth conditions *(67)*.

Growth of *C. botulinum* is inhibited as pH falls from near optimum (7.0) to acidic values. Figure 3 illustrates that by lowering pH from 6.8 to 4.6, a 6-log decrease in growth occurred in a culture medium *(60)*.

Impact of water activity on growth of *C. botulinum*. As water activity is reduced by dehydration or through the

addition of solutes, growth of *C. botulinum* is inhibited, eventually reaching limiting levels. The limiting a_w for growth of group I and II strains of *C. botulinum* is 0.93 and 0.97, respectively *(93)*.

The inhibitory effect of NaCl remains one of the most important factors for controlling *C. botulinum* in foods *(52)*. Salt controls growth of *C. botulinum* at concentrations of 10 and 5% for groups I and II, respectively *(43)*. This inhibitory effect largely is due to the lowered water activity associated with an increase in salt concentration. Riemann *(79)* investigated the effect of NaCl and the initial number of spores on growth of *C. botulinum* type E. Growth of *C. botulinum* was inhibited as salt concentration increased. Figure 4 illustrates that by increasing salt concentration from 0 to 6%, a 5-log inhibition of growth of *C. botulinum* type E strains 04732 and Minnesota occurred in brain heart infusion at pH 7.0 and 30°C *(60, 79)*. Although not explicitly reported, similar results were found for *C. botulinum* types A and B *(79)*.

Impact of nitrite on growth of *C. botulinum*. Nitrite is a common preservative used to control *C. botulinum* spores in meat products. Inhibitory levels of nitrite are 160 to 300 $\mu g\ g^{-1}$ for group I strains and 50 to 160 $\mu g\ g^{-1}$ for group II strains *(93)*. Nitrite delays but does not necessarily prevent outgrowth of *C. botulinum*. Nitrite is depleted more rapidly at low pH or high storage temperature *(52)*. The effectiveness of nitrite is dependent upon complex interactions among pH, NaCl concentration, spore inoculum level, spore injury, storage conditions, and the composition of the food *(52)*.

Figure 5 illustrates that by increasing nitrite concentration to 150 ppm, a 6-log decrease in growth occurred in liver sausage *(42)*. Figure 6 illustrates that with a nitrite concentration of 150 ppm, an 8-log decrease in growth occurred in shelf-stable canned cured luncheon meat with a thermal process of F_o 0.6 and a brine concentration of 4 to 4.5% *(76)*.

Impact of spore injury on growth of *C. botulinum* in the presence of salts. Ingram and Roberts *(48)* explored the effect of injury on *C. botulinum* spores. Spores were heat treated at 95°C and plated on a reinforced clostridial agar (RCA)–bicarbonate medium with and without 2% NaCl and
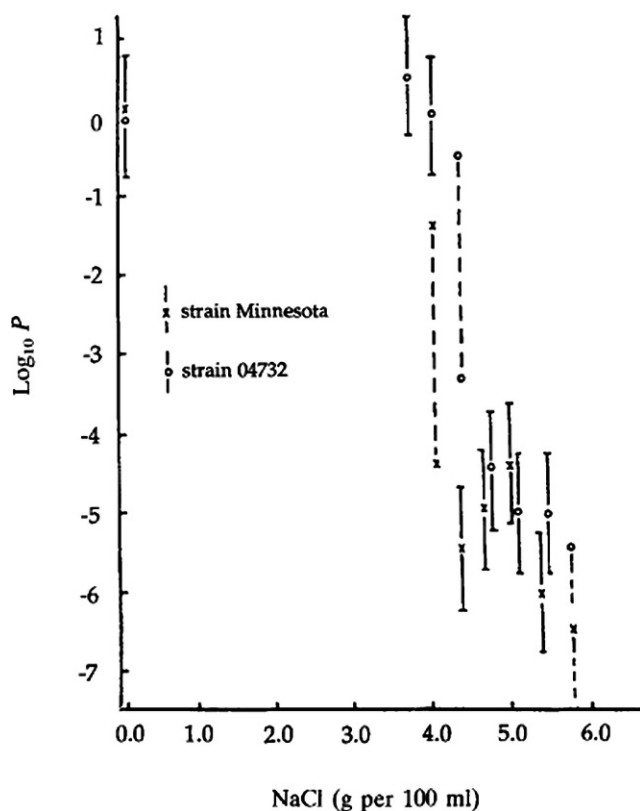
FIGURE 4. *Effect of NaCl concentration on growth of* C. botulinum *type E spores in brain heart infusion, pH 7.0, incubated at 30°C for 2 months. Strains are 04732 (○) and Minnesota (×); bars represent 95% confidence intervals. From Lund (59) as modified from Riemann (79). Copyright held by Chapman and Hall Ltd., London, UK.*
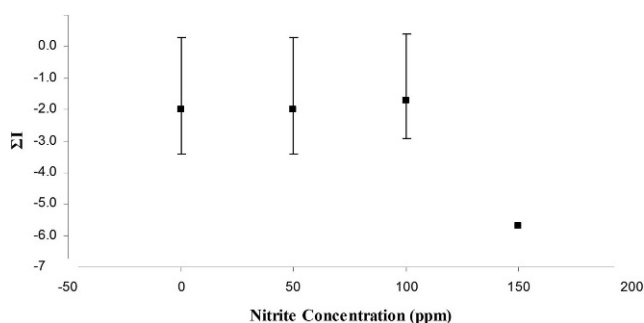

FIGURE 5. *Effect of nitrite concentration on growth of* C. botulinum *type A and proteolytic type B spores in liver sausage (pH 6.1, 2.10% NaCl, a_w of 0.963, 3.7% brine, 53.2% H_2O) after incubation for 1 week at 27°C. Squares indicate experimental results; bars are 95% confidence intervals. Modified from Hauschild et al. (45). Reprinted with permission from the* Journal of Food Protection. *Copyright held by the International Association for Food Protection, Des Moines, IA. A. H. W. Hauschild, R. Hilsheimer, and G. Jarvis, Health and Welfare Canada; D. P. Raymond, Food Production and Inspection Branch, Agriculture Canada.*

confirmed that growth of *C. botulinum* is often controlled by a combination of inhibitory factors because combinations of control measures can be more restrictive to growth and toxin production than is each factor alone *(52, 97)*. As illustrated in Figure 5, even at low concentrations of nitrite, growth of *C. botulinum* is inhibited by approximately 2 log units because of the addition of other control measures, such as water activity and NaCl concentration. In shelf-stable canned cured meats (see Fig. 6), many factors, including mild heat treatment, water activity, and nitrite concentration, result in inhibition of *C. botulinum* growth *(76)*. Figure 8 illustrates combinations of pH and NaCl that result in no toxin production from 10^6 *C. botulinum* type E spores in brain heart infusion at pH 7.0 when the salt concentration was higher than 1.5 and 2.0%. Combinations of pH, moisture, NaCl, and disodium phosphate (Na_2HPO_4) can control growth of *C. botulinum* in processed cheese spreads *(96)*. Figure 9 illustrates the boundary that separates the combinations that did not result in growth and toxin production and those that did after inoculation with 10^4 spores.

Impact of GMPs in prevention of incipient spoilage. The seminal U.S. GMP regulation (21 CFR 128b) for preventing the growth of *C. botulinum* in canned foods was first published in 1973; FDA GMP regulations for low-acid canned foods are now covered under the regulation 21 CFR 113 *(108)*. The Codex Alimentarius Commission adopted the *Recommended International Code of Hygienic Practice for Low and Acidified Low Acid Canned Foods* (CAC/RCP 23-1979) in 1979 and subsequently amended the code in 1989 and 1993 (Rev. 2) *(21)*. The USDA published the FSIS food canning regulations for meat (9 CFR 318.300) *(101–103)* and poultry (9 CFR 381) *(104)*. These recommendations and regulations currently establish procedures and practices that are intended to provide for the effective control of *C. botulinum* in low-acid canned foods. The documents outline critical aspects of the process, especially
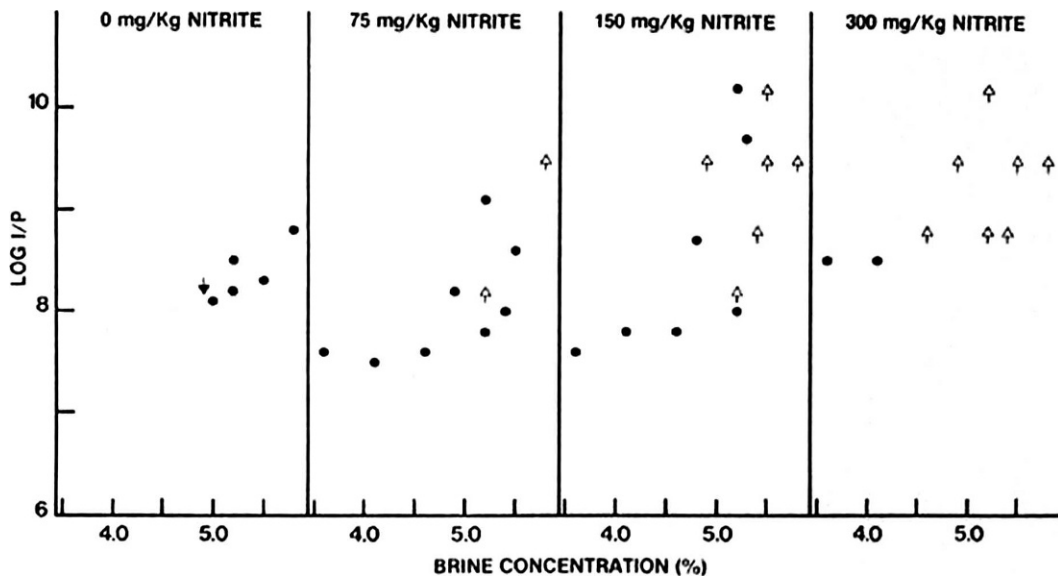
200 ppm of $NaNO_2$. The curves shown in Figure 7 indicate that spore injury and destruction occurred at a predictable rate, D_h, which can be explained by the traditional D-value concept. The $D_{95°C}$- and D_h-values calculated from this plot were 25.1 and 14 min, respectively. Spores that were "killed" by the thermal treatment did not grow when transferred to growth medium. Similarly, spores injured by a sublethal treatment did not grow in the presence of NaCl and $NaNO_2$; however, injured spores would presumably grow if not inhibited by the salts.

For this type of hurdle process, where D_h exists, spore log injury (SLI) of *C. botulinum* spores in cured meats can be quantified by equation 10:

$$SLI = SLR\left(\frac{D_{95°C}}{D_h} - 1\right) \qquad (10)$$

However, to apply the data produced by Ingram and Roberts *(48)*, one must assume that the ratio of $D_{95°C}$ to D_h at 95°C is constant and therefore also applies at 121°C, the typical process temperature for this product. The accuracy of this assumption depends on the activation energy (or z-value) for the injury transformation. The accepted z-value for the inactivation is 10°C. This SLI level will be effective as long as NaCl and $NaNO_2$ are present in the correct concentrations.

Impact of a combination of factors. Extensive research and years of manufacturing experience have

FIGURE 6. *Number of* C. botulinum *spores required for one spore to grow out and produce toxin in shelf-stable canned cured luncheon meat heat processed to ~0.6 F_o. Finite values (●), greater than values (↑), and less than values (↓) are shown. From Hauschild (42) as modified from Pivnick and Petrasovits (76). Reprinted courtesy of* Food Technology *magazine, Institute of Food Technologists.*

those related to thermal preservation methods, which must be controlled if the performance criteria and performance objectives are to be realized. The documents establish a framework for managing the process to ensure the delivery a specified outcome.

These documents were for the most part promulgated in response to illness outbreaks involving *C. botulinum* in 1963 and 1971. In both cases, the actionable cause of the process failure was determined to be a failure by employees of the establishment to thermally treat the food sufficiently to mitigate or inactivate botulinal spores. These incidents reveal that low-acid foods intended for thermal processing in hermetically sealed containers represent a significant risk to the public health when they are not properly heat treated. The canning industry must assume that all raw materials, low acid and otherwise, are contaminated with botulinal spores. Botulism outbreaks have been reported in a wide variety of

foods, including smoked fish, cured meats and sausages, canned fish, canned vegetables, and pickled produce.

Without adherence to GMPs, any process, no matter how well designed, will become out of control. Processors must assume that improper handling of raw food materials during processing will give rise to an increase in the numbers of spores as a consequence of their germination in the food system and thus will result in toxin production. When establishing a low-acid canned foods process, numerous process and product factors must be understood, including but not limited to

- fill weight,
- drain weight,
- headspace,
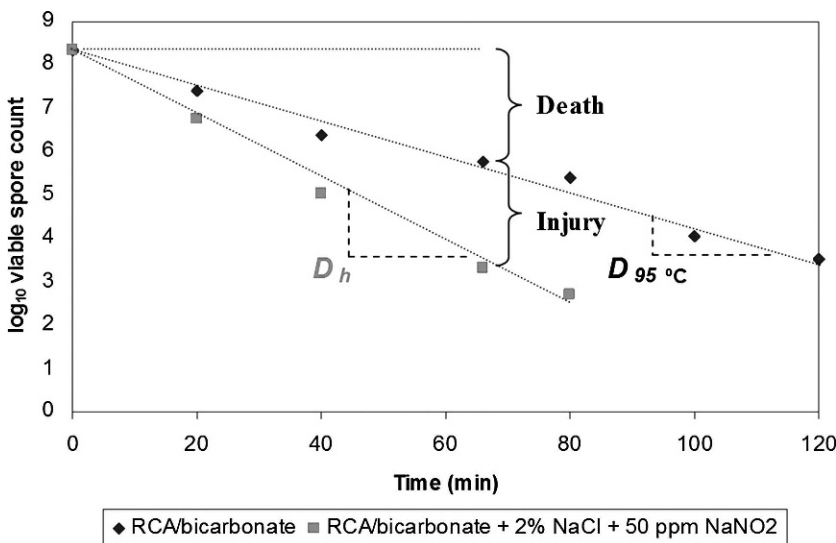- product initial temperature,
- venting schedules,

FIGURE 7. *Effect of NaCl and NaNO$_2$ in an RCA-bicarbonate medium on the reduction of number of* C. botulinum *spores capable of surviving treatment at 95 °C and subsequent growth. Adapted from Ingram and Roberts (48). Reprinted courtesy of* Food Technology *magazine, Institute of Food Technologists.*
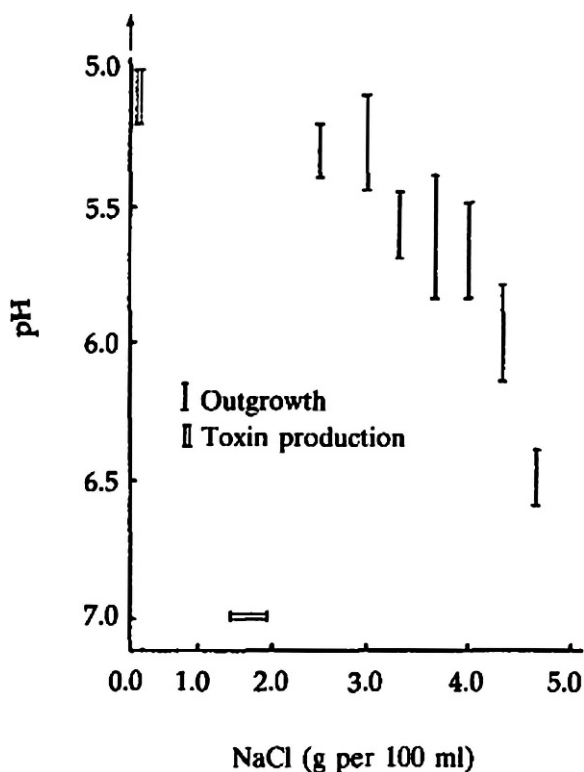
FIGURE 8. *Effect of combinations of pH and NaCl concentration on growth of* C. botulinum *type E spores in brain heart infusion, pH 7.0, incubated at 30°C for 2 months. The intervals indicated by bars represent NaCl concentrations and pH values that prevented toxin growth and toxin formation from 10^6 spores of strain Minnesota. From Lund (59) as modified from Riemann (79).*

FIGURE 9. *Effect of pH, NaCl and Na_2HPO_4 concentration and moisture on growth of* C. botulinum *type A and B spores in pasteurized processed cheese. ○, Zero samples (40 per batch) produced toxin; ×, one or more samples produced toxin during 42 weeks of incubation at 30°C. The solid line indicates the boundary that separates the combinations that did not grow and produce toxin from those that did (96). Reprinted with permission from the* Journal of Food Protection. *Copyright held by the International Association for Food Protection, Des Moines, IA. N. Tanaka, E. Traisman, et al., University of Wisconsin, Madison.*

- come-up time,
- segregation between processed and unprocessed products,
- seam inspection and seal integrity,
- sanitizer concentration in cooling water,
- air and gas filters,
- aseptic shrouding,
- back pressure devices,
- actions on deviations, and
- employee training and supervision.

The inception of low-acid canned foods regulations and recommendations has resulted in a precipitous decline in reported cases of botulism originating from commercial food processing establishments.

The umbrella U.S. GMPs (21 CFR 110) *(105)* and good hygienic practices *(21)* have long been recognized as effective measures for preventing the outgrowth of undesirable microorganisms in food processing operations. The collective contribution of the activities broadly contained in these programs directly impact food safety. Fundamentally, the regulations and recommendations recognize the ubiquitous nature of microorganisms and that controlling dangerous bacteria in processing operations is critical to achieving public health objectives.

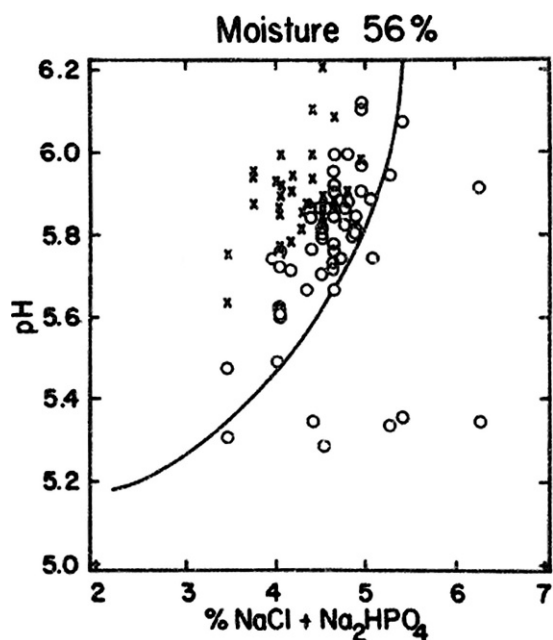GMPs are recognized by the FDA and the Codex as programs and procedures that form the minimum basis for the sanitary production of food products. The framers of these regulations and recommendations recognized the importance of sanitary design and construction of facilities and equipment for preventing the proliferation of both spoilage and pathogenic microorganisms in food processing establishments. Ultimately, the GMPs are focused on preventing incidental or direct contamination of foods held in storage or the other various unit operations involved in food processing. Toward this end, the regulations and recommendations specify materials of construction for floors, walls, and ceilings and for processing equipment. The framers also recognized the potential for workers in food processing operations to contribute to product adulteration with dangerous pathogenic microorganisms. The intent of the umbrella GMPs is to maximize the effectiveness of cleaning and related activities for reducing the background contamination and the reintroduction of hazards. GMPs give consideration to the flow and movement of raw materials across the expanse of the manufacturing supply. The documents specify, for example, control over temperature, moisture, and other environmental conditions that may promote or foster the outgrowth, proliferation, and reintroduction of undesirable microorganisms at the various unit operations of the overall process.

Similarly, GMPs recognize the importance of equipment construction, design, and installation for achieving performance criteria and performance objectives. The GMP regulations require that all food contact surfaces be constructed from smooth, impervious materials and recom-

mend that product contact surfaces and product contact zones of the equipment be protected against incidental contamination. These regulations also specify that process plumbing (e.g., pipes, valves, strainers, and flow control devices) and process monitoring instrumentation must not be installed in manner that will give rise to stagnant product within the confines of the process plumbing. Dead zones with entrained product residues may promote the concentration and proliferation of dangerous microorganisms, becoming focal points for promoting and widely transmitting hazardous bacteria throughout the process.

In terms of ΣI, the total cumulative increase in the microbial hazard, adherence to GMPs and good hygienic practices play a vital role in ensuring attainment of a specified process PO. For example, prevention of excessive delays or downtime in processing operations will minimize the potential proliferation of hazardous microbes. Products in the production process that are held for extended periods at temperatures in a range that is conducive to pathogen growth are at great risk of becoming hazardous because of the potential increase in the numbers of harmful microbes. An increase in the pathogen numbers per unit of food may adversely impact the ability of the preservation step to mitigate the hazard. Proper removal of microbial contaminants from the contact surfaces of processing equipment also will minimize the overall bioburden of foods that come into contact with these surfaces and thus promote the achievement of the desired process performance criteria.

Package integrity and recontamination. The second area of concern for ΣI is the probability of recontamination postprocessing due to package failure. The importance of package integrity for preserving and protecting the safety status of previously processed foods has long been understood. Low-acid canned food regulations and guidance include provisions for inspections of can seams. Despite aggressive, long-standing programs for preventing postprocessing recontamination, several outbreaks have resulted from package failures. Notable among these failures have been incidents involving canned tuna and salmon.

Container leakage due to defective double seams on the cans' ends led to the outbreak associated with canned tuna in 1963 (51). The first outbreak involving commercially canned salmon occurred in the United Kingdom in 1978; the can and seam had been gouged in an undetermined way, and subsequent corrosion led to a small hole at the damage site that created the opportunity for contamination late in the can's history (66). Employees working on the raw fish evisceration lines often placed their wet aprons and gloves on baskets exiting retorts at the end of the day, which may have resulted in transfer of spores to the can. The second outbreak associated with canned salmon occurred in the United States when small triangular holes were punched into the can bodies during the reforming of the can body blank (2, 66, 99). These blanks were then shipped flat to Alaska and reformed into a round can to save on cost. This action ultimately resulted in the cooked salmon being contaminated with nonproteolytic type E *C. botulinum* spores contained in retort cooling water. A concentration of

2 to 5 ppm of available chlorine in cooling water is largely considered adequate for good sanitation control of cooling water; however, at high pH values, inactivation of *C. botulinum* may take many minutes. Nearly 60 million cans from nine canneries were recalled as a result of this incident.

Under present processing conditions, pathogens recovered from canned foods are not typically the result of recontamination. Odlaug and Pflug (69) concluded that the likelihood of postprocessing contamination by *C. botulinum* in canned foods is between 10^{-7} and 10^{-10} per can. However, Stersky et al. (91) reported 154 incidents between 1921 and 1979 that involved food poisoning as a result of postprocess recontamination. Organisms associated with these incidents were *Staphylococcus aureus*, *Salmonella*, *C. botulinum*, and *Clostridium perfringens*. The foods associated with these outbreaks were meat, vegetables, and fish.

Gaps in the literature. Many questions exist regarding the likelihood of a particular load of *C. botulinum* spores producing toxin under conditions on the verge of being inhibitory. For example, a common assumption is that at higher pH values (i.e., between 6 and 7), 100% of *C. botulinum* spores will germinate in a food. At pH values below 4.6, the assumption is that no strains of *C. botulinum* will germinate, grow, and produce toxin. To answer questions related to the establishment of an appropriate treatment, it may be necessary to know the likelihood of a particular inoculum of *C. botulinum* spores resulting in toxin formation near boundary levels of intrinsic properties.

Recommended research required to fill gaps. The following list identifies recommended research to fill the gaps identified.

- Additional studies should be designed to test boundary conditions of intrinsic properties of foods such as pH, water activity, and NaCl combinations to obtain probabilistic values for use in FSO evaluations of a process and to expand the applicability of the FSO concept to commercially sterile foods.
- The *D*-value for spore injury at 95°C has been assumed to be constant and therefore to apply at a sterilization temperature of 121°C. Additional research is needed to confirm that this assumption is valid by determining the *z*-value for spore injury.
- Empirical histograms of the resistance of the bioburden to lethal agents of interest should be constructed as a potential tool to develop inactivation cycles. One example of this approach is the methods supported by the International Organization for Standardization (ISO; Geneva, Switzerland) for radiation sterilization. In that case, tables based on the empirical ''standard distribution of resistances'' were developed that present the radiation dose required to reach a certain sterility assurance level based on the bioburden (ANSI/AAMI ST32-1991, ISO 11137-1994, and ISO 13409-1996).

Economic spoilage considerations. In addition to threats to food safety, processors consider spoilage microorganisms when designing processes to generate

FIGURE 10. *Effect of individual control measures and measures in combination on growth of* C. botulinum *in canned beans, shelf-stable canned cured luncheon meat, pesto, and pasteurized processed cheese spread.*
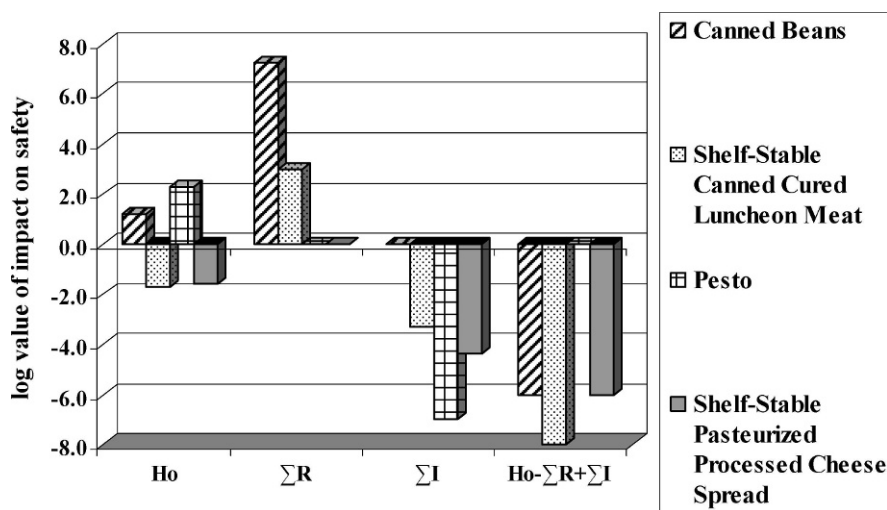
commercially sterile products. A process more stringent than that needed to reach the safety target may be chosen to eliminate spoilage organisms when such organisms are more resistant than the target pathogen. Thus, many thermal processes applied to low-acid canned foods involve temperatures much higher than those necessary to inactivate spores of *C. botulinum*. Processes employed often are based on heating studies using *Clostridium sporogenes* (PA 3679), a mesophilic PA that is more heat resistant than *C. botulinum*. Because of this higher heat resistance, many processes applied to obtain shelf-stable foods are approximately two to three times more severe than those necessary to destroy *C. botulinum* spores. Low-acid canned foods are traditionally processed to provide products with a long shelf life. The principles described in this report can be applied to develop processes that control spoilage and thus yield commercially sterile products. In such a case, the FSO process can be repeated using the initial bioburden, H_0, of a particular spoilage spore former as the target instead of *C. botulinum*. The resistance characteristics and the probability of outgrowth must be determined using the specific values for the target spore former.

ILLUSTRATIVE EXAMPLES

The examples that follow include products well established in the marketplace and illustrate how these products, which have been safely produced over a long period, fit into the risk management framework. Examples were chosen to illustrate how various performance criteria can be met. A variety of preservation conditions and approaches to establishing commercial sterility are presented. The products highlighted in this section are canned beans, shelf-stable canned cured luncheon meat, pesto sauce, and pasteurized processed cheese spread. Figure 10 summarizes the impact of individual elements and the total contribution of control measures for each product example. The ΣR term is presented graphically as a positive log reduction. The safety of canned beans relies solely on the reduction of the hazard achieved by thermal processing, whereas the commercial sterility of processed cheese and

pesto sauce relies primarily on the inhibitory effects achieved through product formulation. Cured canned ham safety relies on low initial levels of contamination, mild thermal processing, and formulation of the product.

For each of these example products, the contributing control measures that determine product safety have been developed into a conceptual framework and interpreted in terms of achieving a given FSO. The intent of these examples is to illustrate the principles of the risk management framework, not to specify target FSOs. Therefore, for each of the products two scenarios are outlined to better illustrate the impact of particular control measures. The detailed data for each deterministic example are presented in Table 5 and are referred to where appropriate in the discussion. In addition to the simple deterministic illustrations, for canned cured luncheon meat a stochastic approach to analysis is provided to illustrate the influence of process variation. Membré and van Zuijlen *(61)* used a probabilistic approach to determine the required thermal process for an ambient stable soup product heated in a continuous ultrahigh-temperature line; however, unlike the analysis provided here for shelf-stable canned cured luncheon meat, this work did not incorporate any contribution by the ΣI term or inhibitory effects.

Canned beans. Canned beans is a product for which it is reasonable to assume that *C. botulinum* spores will be present at a significant level in raw materials, and the conditions of this product are favorable for outgrowth of any surviving or recontaminating spore and for toxin formation during distribution of the product. Vegetables support the germination, outgrowth, and toxin production of group I (proteolytic) strains *(68)* and group II (saccharolytic) strains *(13)* of *C. botulinum,* and sometimes vegetables with *C. botulinum* toxin have normal or near normal odor and appearance. This product category relies almost exclusively on thermal processing and the prevention of recontamination to provide a safe product with respect to *C. botulinum*. In addition to *C. botulinum* spores, canned vegetables are likely to contain spores of thermophilic and mesophilic spoilage microorganisms. Although thermophiles are not

TABLE 5. *Risk for select commercially sterile foods*

Product	H_0 (log [N_0/unit])	ΣR (log [N_0/N_R])	ΣI (log [N_I/N_0'])	$H_0 - \Sigma R + \Sigma I \leq$ FSO (log [N/unit])	Control measures
Canned beans	1.2	7.2	0.0	−6.0	F_o 1.3
		10.2	0.0	−9.0	F_o 1.9
Shelf-stable canned cured	−1.7	3.0	−3.3	−8.0	F_o 0.6, pH 7.0, NaCl 4.5%, nitrite 150 ppm *(76)*
luncheon meat		3.0	−4.3	−9.0	F_o 0.6, pH 6.5, NaCl 5.5%, nitrite 150 ppm *(76)*
Pesto	2.3	0.0	−7.0	>−4.7	pH 4.6 *(59)*
		0.0	−2.0	0.3	pH 4.75 *(59)*
Shelf-stable pasteurized	−1.6	0.0	−4.4	−6.0	pH 5.6, NaCl+Na$_2$PO$_4$ 4.5%, moisture 56% *(95)*
processed cheese spread		0.0	−4.4	−6.0	pH 6.0, NaCl+Na$_2$PO$_4$ 5.0%, moisture 54% *(95)*

pathogenic, they are generally more heat resistant than mesophiles and may cause spoilage under harsh storage conditions. For example, in tropical climates or in storage facilities that can become very hot, abnormally high storage temperatures may result and lead to thermophilic spoilage inside cans. Because many spoilage spores are likely to be more frequently associated with vegetables and are more resistant than *C. botulinum* spores, canning processes for vegetables often are established under conditions that far exceed those required to inactivate spores of *C. botulinum*.

Controlling initial levels in canned beans. Because vegetables often are in contact with the soil, they are easily contaminated with spores of *C. botulinum*. Hauschild et al. *(44)* found 15 *C. botulinum* spores per 100 g of unwashed mushrooms and 41 *C. botulinum* spores per 100 g of washed mushrooms. Solomon and Kautter *(85, 86)* isolated *C. botulinum* from onion skins and fresh garlic clove skins, and Solomon et al. *(87)* isolated *C. botulinum* type A from the outer leaves of fresh cabbage. Concern that modified atmosphere packing of vegetables might provide an anaerobic environment conducive to growth of *C. botulinum* led to examination of 1,118 samples of vegetables packed in 454-g lots under modified atmospheres *(56)*. The incidence of *C. botulinum* spores was 0.36%. In their survey of vegetables across the United States, Meyer and Dubovsky *(62)* found that string beans samples had the highest percentage of cultures positive for *C. botulinum*. From these limited data on the incidence of *C. botulinum* spores on vegetables, a level of 0.036 spore per g was used to establish the H_0. For a unit size of 454 g, this equates to an N_0 of 16.3 spores per unit. An H_0 of 1.2 log CFU per unit was calculated using equation 2 as a conservative deterministic estimate for illustrative purposes and is shown in Table 5.

Reducing levels in canned beans. Retorting time and temperature schedules appropriate to the product and type and size of the container are essential for production of a commercially sterile product. These schedules must be developed by an individual with extensive training in the area of thermal processing. The National Food Processors Association's Bulletin 26-L *(65)* recommends a minimum 5.3-min F_o process for canned beans in heavy sauce (with or without pork) to render the product commercially sterile. The assumption is that all of the necessary steps involved in the preretorting process have been completed before

retorting begins. The retorting step is assumed to deliver an effective treatment equivalent to heating the product at 121°C for 5.3 min. The public health target of concern in this case is *C. botulinum* spores. The $D_{121°C}$ for *C. botulinum* is 0.21 min in phosphate buffer. There are no attenuators within the beans to affect the destruction rate of *C. botulinum*. The SLR for this process is

$$\text{SLR} = \frac{F_o}{D_{121°C}} = \frac{5.3}{0.21} = 25.3 \quad (11)$$

The log reduction for the pathogen of concern that result from this process far exceeds any expected FSO for this type of product. However, in the worked example the authors explored the treatment necessary to achieve an FSO of −6.0, where the probability of 1 unit supporting the growth of *C. botulinum* is at least 1 in 1 million. To achieve this goal, the thermal process would need to deliver at a minimum the equivalent of a 7.2-log reduction. If the required FSO were −9.0, where the probability of 1 unit supporting the growth of *C. botulinum* is at least 1 in 1 billion, then the thermal process would need to deliver the equivalent of a 10.2-log reduction. These values for ΣR are given in Table 5.

Preventing an increase in levels in canned beans. Most canned vegetables are low acid because their pH is above 4.6 and are thus likely to support the outgrowth of surviving *C. botulinum* spores. The assumption was that the conditions of the product did not have any inhibitory effect on growth and that any surviving or recontaminating spore would be capable of growth in this product. Thus, the log ratio of the number spores capable of growth per unit of the product, N_I, to the number of viable spores present at the implementation of the control measure per unit of product, N_0' is 1 and ΣI is equal to zero on the log scale.

Summary of performance criteria to achieve an FSO for canned beans. For this simple deterministic example, where a reduction in spore numbers is the only control measure, given an H_0 of 1.2, an FSO of −6.0 or −9.0 can be achieved by a process that delivers a 7.2- or 10.2-log reduction, respectively (Table 5). Thus, with greater reduction in levels, a more stringent target FSO can be achieved. Using these performance criteria, the expected probability is less than one spore capable of growth in 1 million units (FSO of −6.0) or 1 billion units

(FSO of -9.0). Variability in the incoming bioburden and in processing will affect the ability of the processor to achieve the target FSO. The effects of variability are illustrated in a stochastic model developed for shelf-stable canned cured luncheon meat below.

Pesto sauce. Pesto, an Italian-style pasta sauce, has been implicated in foodborne botulism cases in China, India, and Italy *(1)*. The outbreaks are reported to have involved a *Clostridium butyricum* strain that produced botulinum toxin type E. Investigators of the outbreak reported outgrowth with toxin production by *C. butyricum* within 5 days in pesto (pH $\geq$ 4.8) held at 25°C. These researchers also reported growth and toxin production in 15 days when the product's temperature was held constant at 12°C.

Pesto is typically formulated with the following ingredients: basil, garlic, pine nuts, parmesan cheese, and edible oil (canola or olive). The ingredients of this product, especially the garlic and basil, can be expected to contain relatively high levels of botulinal spores. This sauce is most frequently formulated and packaged for sale as a refrigerated product. However, ambient stable acidified versions of the product can be found in the distribution chain.

The only notable difference in the product formulation between the refrigerated and ambient stable version is the addition of an acidulant. Citric acid and/or lemon juice are frequently used for acidifying the ambient stable version of the product. The objective of acidification is to reduce the final equilibrated pH of the product to below 4.6. Acidification negates the requirement for providing a thermal process with lethality sufficient to eliminate the *C. botulinum* spores residing in the food. Typically, the pH is in the range of 3.8 to 4.4 for commercially available pesto sauce. The thermal processing for a pesto sauce that has been acidified might range from mild pasteurization (2 min at 72.2°C [162°F]) to a more rigorous process involving heating the food to 90°C and holding for 5 min before filling the containers. The application of thermal processes for ambient stable pesto sauces varies widely. Frequently, decisions related to the level of thermal processing are a function of the organoleptic properties of the finished product.

Pesto sauces—refrigerated or ambient stable—typically exhibit a marked separation of the oil and aqueous phases. For the mild thermal processes that are used for stabilizing the ambient stable pesto product to be effective, acidification of the aqueous phase must be sufficient to not allow an increase in product pH during the course of normal storage or distribution. The incoming *C. botulinum* spore loads on the basil, garlic, and other constituents have not been reduced by the applied thermal treatment. Consequently, the only barrier or hurdle preventing spore germination, outgrowth, and toxin production is the presence of the acidulant in the aqueous phase of the sauce at the appropriate concentration.

Control measures for pesto sauce. Formulation and formula control are critical for preventing the outgrowth of spores intrinsic to this sauce. The proportions of basil and garlic must be properly controlled, especially where the stabilization methods are dependent on acidification. Adequate and reliable acidification of the vegetable components is difficult to achieve when these components exceed 40 to 50% by weight of the formula. The buffering capacity of both the added cheese and the pine nuts also must be considered. Whole pine nuts may be problematic because they are very slow to become acidified. Consideration also must be given to the impact on water activity that results from using individually quick frozen ingredients, especially for those processes that rely on water activity and heat alone for stabilizing these products.

Anniballi et al. *(1)* reported that the *C. butyricum* strains used in their work with pesto sauce were capable of outgrowth and toxin production at ambient temperature and at abusive refrigeration temperatures.

Controlling initial levels in pesto sauce. Three of pesto's ingredients, sweet basil, garlic, and pine nuts, often are come into contact with the soil and may be easily contaminated with spores of *C. botulinum*. *C. botulinum* spores have been isolated from onion and fresh garlic clove skins *(85, 86)*. Hauschild et al. *(44)* found up to 2,100 *C. botulinum* type B spores per kg of raw mushrooms. Notermans et al. *(67)* estimated the incidence of *C. botulinum* at between 0.8 and 1.6 spores per kg. From these limited data, 1 spore per g was taken as a conservative maximum concentration. For a unit size of 200 g, the N_0 would be 200 spores per unit. An H_0 of 2.3 log CFU per unit was calculated using equation 2 as a conservative deterministic estimate for illustrative purposes, as shown in Table 5.

Reducing levels in pesto sauce. Ambient shelf-stable pesto is typically pasteurized for 2.0 min at 72.2°C (162°F). The thermal process used in the manufacture of these products is sufficient to eliminate any vegetative pathogens of concern, such as *Listeria monocytogenes, Staphylococcus aureus, Salmonella,* and *Escherichia coli*; however, the spore-forming bacterial pathogens *C. botulinum* and *B. cereus* can survive the thermal process. Thus, the log ratio of the number of viable spores present at the implementation of the control measure per unit of product, N_0', to the number spores capable of growth per unit of the product following the reduction, N_R, is 1 and ΣR is equal to zero on the log scale.

Preventing an increase in levels in pesto sauce. Safety of pesto with respect to *C. botulinum* results from formulation that prevents spore germination and outgrowth. Acidification reduces the final equilibrated pH of the product to below 4.6. Typically, the pH is in the range of 3.8 to 4.4 for commercially available pesto sauces. As shown in Figure 3, at a pH below 4.6, greater than a 7-log inhibition is achieved *(60)*. However, pesto sauce relies heavily on GMPs. When pesto is improperly formulated, achieving the ΣI may mean the difference between a safe product and a food that is unfit for human consumption. For instance, if final equilibrium pH is 4.75, ΣI will be approximately -2.0.

Summary of performance criteria to achieve an FSO for pesto sauce. As seen in Table 5, given an H_0 value of 2.3, a ΣR value of 0.0, and a ΣI value of -7.0, the combined effect, $H_0 - \Sigma R + \Sigma I$, under target conditions would be less than -4.7 log spores per unit capable of growth. However, at equilibrium pH 4.75, $H_0 - \Sigma R + \Sigma I$ would be approximately 0.3; thus, one would expect that approximately two spores per unit would be capable of growth. Under these conditions, the product would clearly be a risk to public health if distributed for consumption.

Shelf-stable pasteurized processed cheese spread (SSPPCS). Three classes of processed cheese products are generally recognized: pasteurized processed cheese, pasteurized cheese food, and pasteurized processed cheese spread (27). ''Process(ed) cheese and spreadable process(ed) cheese are made by grinding, mixing, melting and emulsifying with the aid of heat and emulsifying agents one or more varieties of cheese, with or without the addition of milk components and/or other foodstuffs'' (24). In the United States, the Code of Federal Regulations recognizes several categories of processed cheese (21 CFR 133 (109)), defined primarily by cheese type, moisture, and milk fat content.

In pasteurized processed cheese spread, some of the natural cheese solids are replaced with skim milk, whey, or cream, and this food may contain sweetening agents and gums. The moisture level is about 50 to 60%, the pH is in the range of 4.1 to 6.0, and the a_w is 0.93 to 0.95. These products also may contain delta-gluconolactone for lowering pH (27).

SSPPCSs have been commercially produced for more than 40 years and have an excellent safety record. Botulism outbreaks were associated with shelf-stable processed cheese spread in 1951 (United States) and 1974 (Argentina), but the pH and water activity of the spreads in both instances were considered sufficient for C. botulinum growth. In the U.S. outbreak, the pH was 5.9, and in the Argentine outbreak the pH was 5.6 to 6.1 and the a_w was 0.97 (27).

Refrigerated processed cheese products do not require the same formulation controls as do shelf-stable products. The safety of the shelf-stable products is derived instead from cooking and filling to control vegetative pathogens combined with refrigeration as the primary barrier to spore germination and outgrowth. In refrigerated products, formulation provides a 'secondary barrier' against short periods of temperature abuse. Refrigerated products are not considered in this discussion.

Control measures for SSPPCS. To ensure the safety of SSPPCSs, hurdles are used in their formulation. Preservatives and other factors at subinhibitory levels may not inhibit the growth of pathogens when used alone but in combination may have an increased antimicrobial effect (54). A hurdle approach that relies on a number of preservation (control) factors such as pH, moisture, water activity, sodium phosphate or polyphosphates, sodium citrate and/or nisin, or other antimicrobials is currently commercially used to produce these products. The effec-

tiveness of any single control measure is dependent upon a complex interaction with other control measures, and shelf-stable processed cheeses are complex products with respect to the inhibitory factors that act on C. botulinum. Although the hurdle approach is complex, it allows for some flexibility in product formulation; however, only a small change in the level of one factor (e.g., pH), can lead to a microbiologically unsafe product if compensation is not made with another factor (e.g., NaCl).

Tanaka and others (94, 96) comprehensively studied the effects of NaCl, phosphates, moisture, and pH for achieving safe and stable processed cheese spreads with regard to C. botulinum outgrowth. Recently, this work was expanded, and predictive models for the time to a 100-fold increase in proteolytic C. botulinum in high moisture pasteurized processed cheese spreads were developed (95, 96). If shelf-stable processed cheese spreads are formulated to be safe with respect to C. botulinum, they should also be safe with respect to B. cereus. For example, the inhibitory level of one of the key hurdles in processed cheese (salt) is lower for B. cereus than for proteolytic C. botulinum strains (50).

Controlling initial levels in SSPPCS. The contamination of C. botulinum in milk is estimated to be about 1 spore per liter (27). Franciosa et al. (35) suggested that a sample size of at least 50 g is needed to give a realistic estimate of the number of organisms present. Based on the results obtained from an unopened can of infant formula, Brett et al. (11) estimated that a 200-g sample is necessary for a 95% chance of detecting a single C. botulinum spore. From these limited data, 0.1 spore per kg was the estimated level. Thus, for a 224-g package, an H_0 of -1.6 log CFU per unit was calculated as a deterministic estimate for illustrative purposes and is given in Table 5.

Reducing levels in SSPPCS. Typically, temperatures not less than 90°C during cheese melting and temperatures not less than 76°C during container filling are used in manufacture of these products; these temperatures are well below those required for inactivation of proteolytic C. botulinum spores. The thermal process used in the manufacture of these products is sufficient to eliminate any vegetative pathogens of concern, such as L. monocytogenes, S. aureus, Salmonella, and E. coli. However, the spore-forming bacterial pathogens C. botulinum and B. cereus can survive this thermal process. Thus, the log ratio of N_0' to N_R is 1, and the contribution to ΣR for C. botulinum spores by this mild heat treatment is zero.

Preventing an increase in levels in SSPPCS. Because no reduction in levels of C. botulinum spores is achieved from thermal treatment alone, the product must be formulated to prevent germination and outgrowth of these spores. Ter Steeg and Cuppers (98) reported that at typical pH levels (pH 5.5 to 5.8) and when 2.5% sodium phosphate or polyphosphate is used as an emulsifier, the critical a_w for controlling outgrowth is approximately 0.95 for proteolytic C. botulinum spores and approximately 0.97 for nonproteolytic species in ideal growth medium. Decreasing the pH in

processed cheese formulations had a greater impact on outgrowth than did decreasing the water activity. The addition of NaCl had a clear inhibitory effect, whereas the addition of glycerol (nonionic solute) for adjusting the water activity had little effect. Ter Steeg and Cuppers concluded that salts (NaCl) and emulsifying salts can be pooled as total salts in moisture (or brine weight), and 5% is the growth limit for nonproteolytic *C. botulinum* under optimal conditions. Polyphosphates were more inhibitory than citrates. This finding agreed with that of Tanaka et al. *(95),* who observed that increasing the phosphate concentration tended to decrease the rate of toxin development.

Based on the work of Tanaka et al. *(96),* a formulation that prevents the outgrowth of *C. botulinum* spores in shelf-stable processed cheese spreads is a pH of 5.8, a_w of 0.95, 2% NaCl, 2.5% disodium phosphate, and moisture levels of up to 54%. As shown in Figure 9, at a pH of 5.6, a_w of 0.95, 2% NaCl, 2.5% disodium phosphate, and moisture level of 56%, a greater than 4-log inhibition is achieved *(60).* Thus, the value of ΣI under these formulary conditions is -4.4. Similar levels of inhibition can be achieved at pH 6.0, a_w of 0.95, 2% NaCl, 2.5% disodium phosphate, and a moisture level of 54%. If improperly formulated, achieving the ΣI may also be the difference between a safe product and a food that is unfit for human consumption. The same combination of product parameters, with the moisture content raised to 58%, allowed toxin to be produced in tested samples. The addition of nisin for controlling *C. botulinum* in high moisture cheese spreads was reported by Somers and Taylor *(89).* Product criteria that prevented toxin production as reported were 57% moisture, 1.4% disodium phosphate, 1.2% added NaCl, and 250 ppm of nisin. When moisture content was lowered to 54%, a combination of 1.7% disodium phosphate, 0% added NaCl, and 100 to 150 ppm of nisin was effective.

Recontamination can occur in steps subsequent to the thermal process (i.e., during packaging); however, adherence to GMPs, hot filling into packaging, and immediate hermetic sealing can prevent recontamination.

Summary of performance criteria to achieve an FSO for SSPPCS. As shown in Table 5, given an H_0 of -1.6, a ΣR value of 0.0, and a ΣI value of -4.4, the combined effect ($H_0 - \Sigma R + \Sigma I$) under target conditions would be less than -6.0 log spores per unit capable of growth. In other words, the expected probability is less than one spore capable of growth in 1 million units of pasteurized processed cheese spread.

The process must be validated as capable of sufficient lethality to assure absence of vegetative pathogens, and the product must be formulated to assure that germination, outgrowth, and toxin production by *C. botulinum* cannot occur. Safe formulation can be verified either by *C. botulinum* challenge studies or by reference to accepted formulation models, such as that of Tanaka et al. *(96).* A HACCP program must be designed to pay particular attention to process temperature and time and the formulation factors assuring protection against spore outgrowth. For example, with the Tanaka et al. model *(96)* safe formulation

must be demonstrated through proper ingredient addition for all elements in the formulation scheme:

- added salt (NaCl) and phosphate emulsifying salts,
- factors controlling final moisture content, including raw material moisture and added water,
- factors controlling final fat level, including raw material fat content and added major ingredients such as water, and
- added acidulant and final pH.

The HACCP plan should describe how the addition of appropriate quantities will be controlled, what verification procedures (e.g., laboratory testing or process audits) will be used to confirm that the process is under control, and at what frequency this verification is to be done.

Shelf-stable canned cured luncheon meat. Billions of pounds of shelf-stable canned cured meats have been produced in commercial establishments and safely consumed in the United States over the past 70 years. The safety record with regard to foodborne illness is excellent but not perfect. Foodborne outbreaks associated with the consumption of these products have been linked to underprocessing *(100).* The product is formulated with sodium nitrite (78 to 156 ppm), NaCl (3.5%), and phosphate (0.5%), with a pH of 6.0. Data from the literature indicate that *C. botulinum* outgrowth and toxin production are possible in these formulations *(40).* Although the thermal process inactivates vegetative cells, spore-forming pathogens such as *C. botulinum* can survive the thermal process; however, injured *C. botulinum* spores cannot repair and fail to grow and reproduce in the presence of NaCl and $NaNO_2$. Therefore, the microbiological safety of shelf-stable canned cured luncheon meat (SSCCLM) relies on mild heat treatment in combination with the presence of curing salts in the formulation to prevent outgrowth and toxin formation from a low level of indigenous spores. These products are thermally processed in the primary package, so postprocess contamination is prevented when proper GMPs are followed. However, storage at ambient temperatures will not prevent the growth of mesophilic spores. Therefore, these products would favor the growth of spore-forming bacteria, which can survive the processing step and are able to grow in the absence of oxygen at ambient temperature. Toxin production by proteolytic *C. botulinum* is the main concern for the safety of these products.

Controlling initial levels in SSCCLM. Spencer *(90)* concluded from the literature that a low level of spores, <1 spore per g, was necessary for the safety and stability of shelf-stable canned cured meats. Greenberg et al. *(40)* conducted the most comprehensive survey to date on the incidence of mesophilic *Clostridium* spores in raw meat and poultry. Of 19,727 PA spores isolated from 2,358 raw meat samples, only 1 spore was confirmed as *C. botulinum* type C, with a level of 5.33 spores per g. Data from this study indicate a very low incidence (0.042%) and level (0.1 spores per kg) of *C. botulinum* spores in raw meat. Studies tend to report levels between 0.1 and 10 spores per kg *(29).* Genigeorgis *(36)* reported levels not exceeding 4 spores per

kg, and Hauschild (43) tabulated data from a number of reports on raw and semipreserved fish and meats. Based on these data, a level of 0.1 spores per kg and a unit size of 200 g were used to establish the H_0 of -1.7 log CFU per unit given in Table 5.

Reducing levels in SSCCLM. By using equation 12 for a 0.7-min F_o isothermal process corresponding to *C. botulinum* with a $D_{121.1°C}$ of 0.21 min at 121.1°C, the following value for the SLR was obtained:

$$\text{SLR} = \frac{F_o}{D_{121°C}} = \frac{0.6}{0.21} = 2.9 \qquad (12)$$

Therefore, the contribution to ΣR by this mild heat treatment is 2.9.

Preventing an increase of levels in SSCCLM. Canned cured meats receive a minimal heat process ($F_o = 0.2$ to 0.7), which is not adequate to inactivate *C. botulinum* spores. Duncan and Foster (30) found that nitrite enhanced heat injury and was strongly inhibitory at pH 6.0, the normal pH of canned meats. Silliker et al. (81) concluded that the combination of sodium nitrite (>78 ppm), salt, heat injury, and a low level of spores is effective for preventing spore outgrowth in these products.

For this type of hurdle process, the SLI of *C. botulinum* spores in cured meats can be quantified by equation 10. Based on a $D_{95°C}$ of 25.1 min and a D_h of 14 min, with equation 13 SLI can be estimated as

$$\text{SLI} = 2.9\left(\frac{25.1\ \text{min}}{14\ \text{min}} - 1\right) = 2.3 \qquad (13)$$

The contribution to the inhibitory effect, captured by the ΣI term, would be the negative of the SLI (-2.3) for the formulary conditions above.

Summary of performance criteria to achieve an FSO for SSCCLM. Given the low initial bioburden of meat, the mild heat treatment, the inhibitory effect of salts on injured spores, with an H_0 of -1.7, a ΣR value of 2.9, and a ΣI value of -2.3, respectively, the combined effect ($H_0 - \Sigma R + \Sigma I$) under these conditions would be -6.9 log spores per unit capable of growth. Experimental data suggest close agreement with these calculations of ΣR and ΣI (Fig. 6). The probability that one spore will grow and produce toxin is on the order of 10^{-7} with an F_o of 0.6 min, pH 7.0, 4.5% NaCl, and 150 ppm of nitrite (76). Thus, the expected probability would be less than one spore capable of growth in approximately 10 million units of shelf-stable canned cured meat.

INFLUENCE OF NONUNIFORMITY: SSCCLM STOCHASTIC EXAMPLE

The previous deterministic examples are helpful for illustrating the principles of the FSO approach; however, deterministic calculations assume no variability in parameters and are often based on worst-case scenarios. Knowledge of the mean or maximum value is useful, but some measure of population variance is needed for a complete statistical description (10). In this example, the influence of variation in the initial bioburden (H_0), in processing (ΣR), and in formulation (ΣI) on the ability to meet a target FSO for SSCCLM is examined.

Controlling initial levels in SSCCLM. Most researchers have reported *C. botulinum* contamination of meat at levels of 0.1 to 10 spores per kg (29). These estimates are influenced by sample size and bacteriological method. Higher estimates are based on larger samples and more robust methods (58). For this example, variability in spore levels was assumed to be properly represented by a lognormal distribution with a mean of 0.4 spores per kg and a standard deviation of 0.5 spores per kg to realistically capture the low probability of upper estimates of 10 spores per kg (Fig. 11).

Variability in spore level was the only variable explored in this model. Additional complexity and improved approximation could be added to the model by including uncertainty in parameter estimation and spore level. The lognormal distribution of spore level was used to construct a distribution through appropriate unit conversions and MC simulation (@Risk, Palisade Corp., Ithaca, NY) to represent the H_0 value as log spores per unit, which is shown in Figure 12.

Reducing levels in SSCCLM. Two main factors contribute to variability in retort processing: (i) heat distribution, i.e., the ability of the retort vessel to deliver uniform temperature distribution in the heating medium, and (ii) heat penetration, i.e., variability in heat delivery from the container surface to the cold spots of the food product (84). Current methods for calculating lethality assume worst-case conditions and do not consider variability in process parameters; however, lethality is influenced by biological, operational, and physical factors. Most variation is associated with physical factors (113). Consequently, F_o is not a fixed value; rather, it is variable. Smout et al. (84) found that for mostly uniform products such as beans, variation in F_o was caused by nonuniformity in retort temperature, whereas when product nonuniformity was high, the factor was dominant in lethality variation. In these studies of a variety of canned vegetable products, the coefficient of variation in F_o values for heating ranged from 15 to 63%. Longer process times result in increased variation in processing (113).

In some instances, a normal distribution may not be appropriate to describe the variation in lethality (84); however, for this example a normal distribution for lethality was assumed. The target F_o was 0.7 min. Because a relatively short process time is necessary to achieve this target, the coefficient of variation was assumed to be 15%. The mean F_o value was taken to be one standard deviation (0.12 min) from the minimum (0.83 min). The resultant distribution of variation in the delivered process is depicted in Figure 13. The normal distribution of F_o was subsequently used in MC simulations to predict the SLR, and the distribution of the resulting ΣR term is shown in Figure 14.

Preventing an increase in levels in SSCCLM. The microbiological safety of SSCCLM relies on a mild heat treatment to injure spores and on the presence of curing salts in the product formulation. For this example, only the

FIGURE 11. *Estimated lognormal distribution* ($\mu = 0.4$, $\sigma = 0.5$) *of* C. botulinum *spore level (number of spores per kilogram) in meat, where μ is the mean and σ is the standard deviation of ln(s).*

Concentration of *C. botulinum* spores kg^{-1}

—— Lognorm(0.4,0.5)
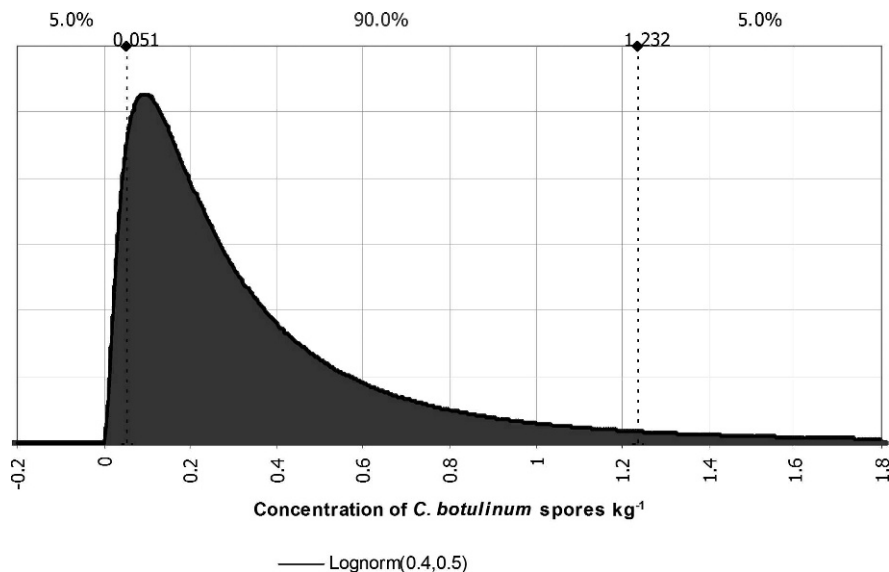
variation associated with the thermal process and spore injury was explored. Additional complexity and improved approximation could be added to the model by including the effect of variability in pH and nitrite concentrations achieved during formulation.

The normal distribution of F_o as depicted in Figure 13 was subsequently used in MC simulations to predict the SLI, and the distribution of the resulting ΣI term is shown in Figure 15.

Summary of performance criteria to achieve an FSO for SSCCLM. MC simulation was used to predict the distribution of the final number of *C. botulinum* spores per unit of SSCCLM that were capable of growth, which is depicted in Figure 16. Results of the model indicate that greater than 95% of the spore population would have a resultant $H_0 - \Sigma R + \Sigma I$ of -6.0, or a probability that 1 in 10^6 units of SSCCLM would contain a *C. botulinum* spore capable of growth. The combined effect of $H_0 - \Sigma R + \Sigma I$ with the deterministic model was -6.9 log spores per unit

capable of growth, which reveals that when the tail of the cumulative distribution $H_0 - \Sigma R + \Sigma I$ is considered, the model is nearly 1 log unit more conservative. The mean (-8.0) and standard deviation (1.09) of the resultant distribution of the final population were in close agreement with the findings of Hauschild *(42)*, who found a degree of protection of 10^{-7} to 10^{-8} per unit under the same formulary conditions.

CONCLUSIONS

To date, thermal processing is the closest thing to a "silver bullet" technology for producing commercially sterile low-acid foods. However, as novel microbial inactivation technologies are developed and refined, there is a growing need for a universal metric that can be used to judge equivalent levels of hazard control stringency to ensure food safety. As discussed in this article, the FSO approach offers such a metric, moving the food supply chain away from the use of prescriptive control measures and toward a risk-based process development

FIGURE 12. *Estimated distribution obtained from Monte Carlo simulation (10,000 iterations) to represent the* H_0 *value as log* C. botulinum *spores per unit of 200 g.*
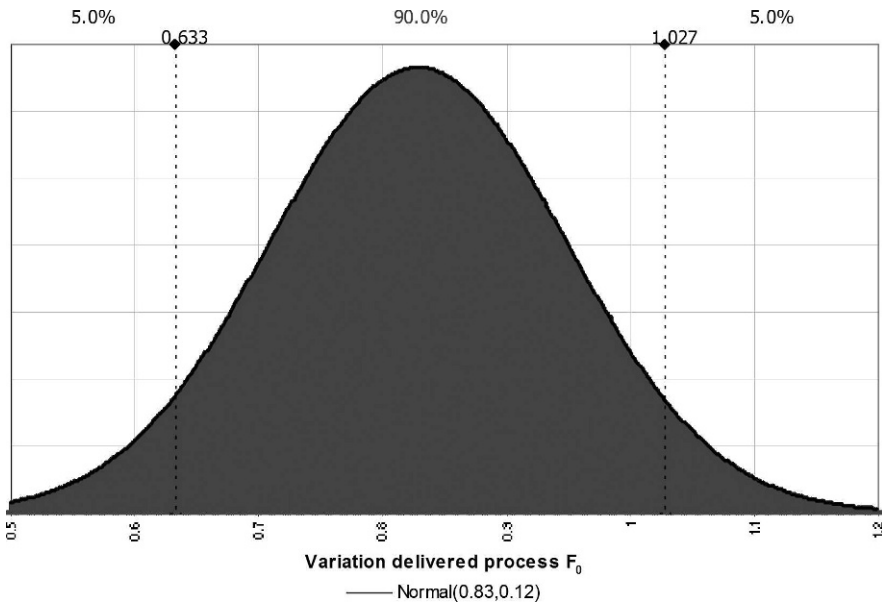
H_0
(log$_{10}$ spores unit^{-1})
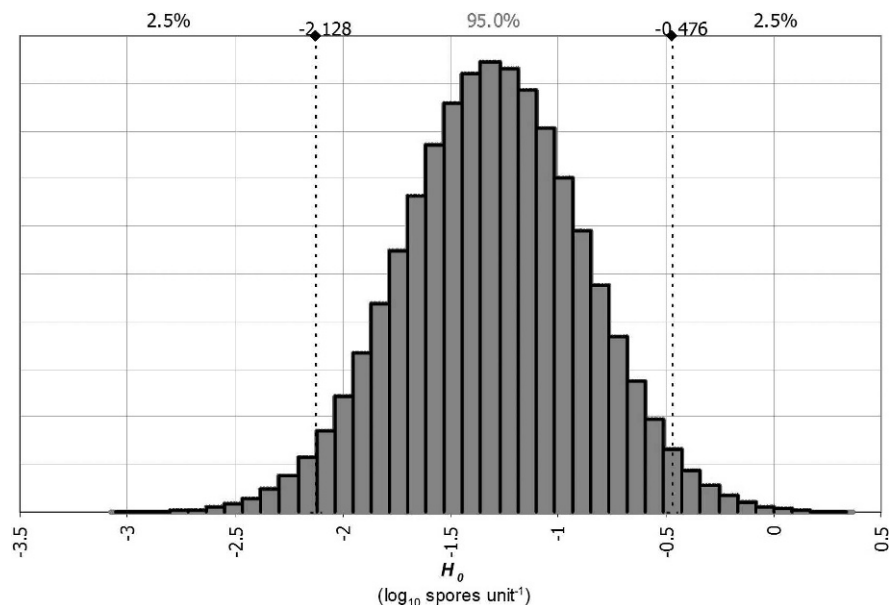
FIGURE 13. *Estimated normal distribution* ($\mu = 0.83$ *min,* $\sigma = 0.12$ *min) of variation in delivered process,* F_o.

framework that can allow flexibility in how a targeted outcome is achieved. Essentially, the FSO risk management concept allows users to evaluate whether food safety processes have an equivalent level of protection by determining whether each method would result in an equal probability that a single unit of commercially sterile food would contain *C. botulinum* toxin. This determination enables food producers, manufacturers, distributors, and handlers to utilize the most effective food safety measures for their individual operations while assuring an equivalent level of hazard control that strengthens control along the entire supply chain.

Although recommendations are presented here for setting an FSO based on severity, risk and likelihood of occurrence for controlling *C. botulinum* toxin in commercially sterile foods, there currently is no universal agreement on the appropriate target FSO. Many of the challenges faced by risk assessors wishing to apply the FSO approach are related to the inherent complexity of the systems described. These issues are related to the need for better microbiolog-

ical data and statistical characterization of variability in unit operations and complex interactions among several control factors, such as pH, water activity, and antimicrobial agents. Often, only a small change in one factor may lead to a microbiologically unsafe food if this change is not appropriately compensated for by changes in another control factor. Nevertheless, ''given sustained attention these and other technical problems are manageable and could be solved'' *(34)*. Further evaluation by an expert panel will be necessary to provide the scientific advice needed to select appropriate control measures that will achieve the desired level of consumer protection against microbial hazards in food.

Ultimately, risk-based process development using the FSO approach offers several key benefits to government, industry, and academic institutions. For government, this approach offers an internationally accepted, science-based approach for assessing public health risk. FSOs can be used to assess whether foods imported from other countries have
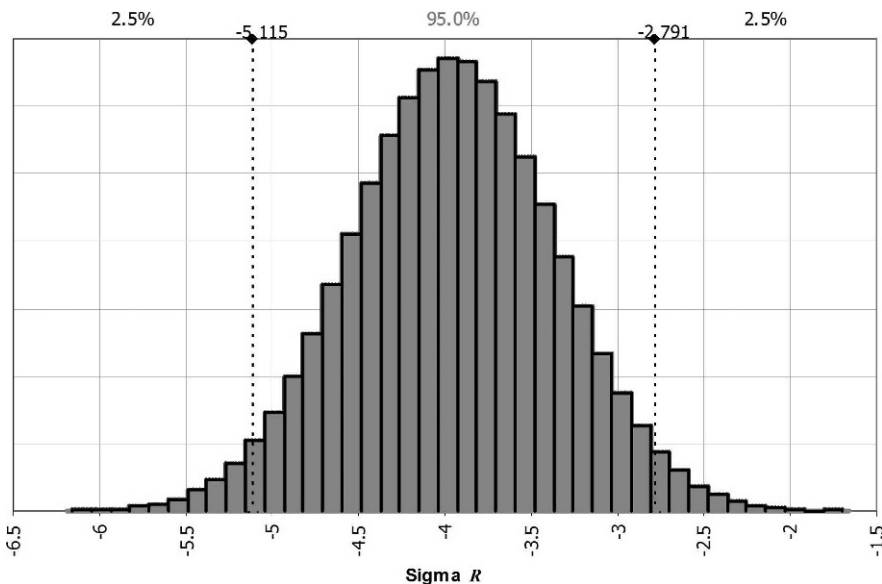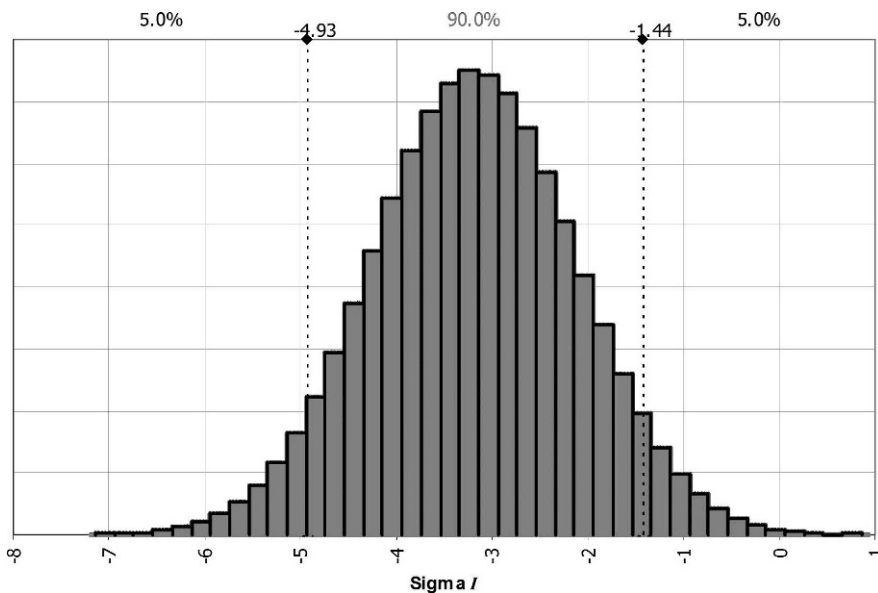

FIGURE 14. *Estimated distribution of the* ΣR *value obtained from MC simulation (10,000 iterations) of SLR calculations.*

FIGURE 15. *Estimated distribution of the* ΣI *value obtained from MC simulation (10,000 iterations) of spore log increase (SLI) calculations.*

been produced in a manner that provides a level of protection equivalent to that required for domestically produced foods. The FSO risk management approach also may be used to assess whether novel processes that utilize single or multiple control measures provide a level of protection equivalent to that of traditional processing methods. For industry, this risk-based process development approach provides a road map for safe innovation and will encourage the development of innovative technologies, such as high pressure processing, pressure assisted thermal sterilization, and pulsed electric fields, which offer food producers, processors, and handlers greater confidence that food safety measures are viable and effective. For academic institutions, this work identifies many opportunities for researchers to make vital contributions to the success of risk-based process development, particularly in the areas of innovative process technologies, mathematical modeling, and preservative-based multiple-hurdle preservation. Cooperative efforts among experts from industry, academia, and government across many disciplines, including microbiology, engineering, manufacturing, and risk assessment, are needed to continue to move forward in the application of risk-based process design. The FSO risk management approach offers all stakeholders a promising avenue by which to take the vital next steps toward a science-based solution that will enhance the safety and quality of the food supply worldwide.

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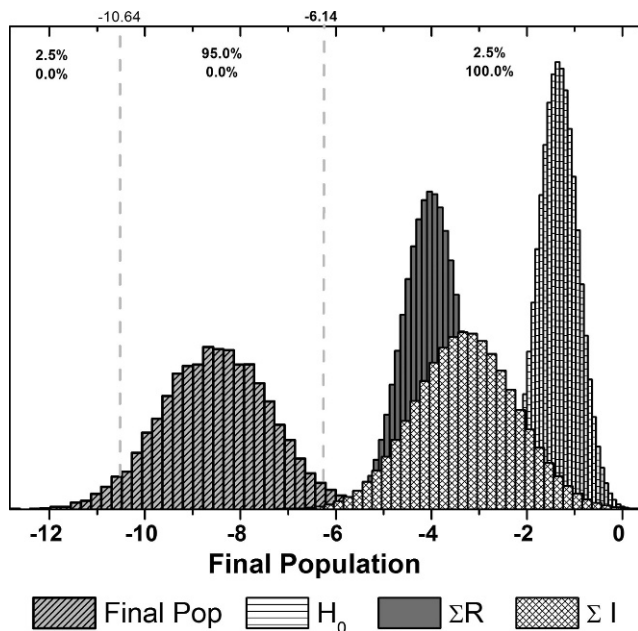
FIGURE 16. *Summary of performance criteria to achieve an FSO including estimated distributions of* H_0 *(log viable spores per unit),* ΣR *(unitless),* ΣI *(unitless), and final population (log spores capable of growth per unit) (normal:* $\mu = -8.0$, $\sigma = 1.09$) *obtained from MC simulation (10,000 iterations).*

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