NATIONAL ADVISORY COMMITTEE ON MICROBIOLOGICAL CRITERIA FOR FOODS

CONSIDERATIONS FOR ESTABLISHING SAFETY-BASED CONSUME-BY DATE LABELS FOR REFRIGERATED READY-TO-EAT FOODS

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Executive Summary

The National Advisory Committee on Microbiological Criteria for Foods (NACMCF, or the Committee) was asked to provide advice on the requisite scientific parameters for establishing safety-based use-by dates for refrigerated ready-to-eat (RTE) foods to help reduce the incidence of foodborne illness.

To address this request, the Committee reviewed the history of the use of date labels, conducted a hazard analysis of refrigerated RTE foods, provided examples of how Safety-Based "Use-By" Date Labels (SBDLs) can be formatted and applied, and answered the specific questions posed to the Committee.

The Committee determined that if the SBDL concept is pursued, *Listeria monocytogenes* is the appropriate target organism for refrigerated RTE foods that support its growth. It is important to note that an SBDL will not prevent illness if the food is heavily contaminated, held at high temperatures, or otherwise abused.

Given the morbidity and high mortality of *L. monocytogenes* infection and the association of *L. monocytogenes* with refrigerated foods, the Committee believes the use of an appropriate SBDL, developed according to the scientific criteria defined herein, could have a beneficial public health impact. Improved epidemiological links between listeriosis and the implicated food could further support this belief. The application of an SBDL for products that support rapid growth of *L. monocytogenes* at the consumer and food handler level, e.g., "use within *x* days" of opening/purchase, may have a positive impact on public health if combined with an effective educational program for temperature control at the consumer level. Research is needed to determine consumers' knowledge, attitudes, and practices in relation to SBDLs (refrigeration times and temperatures) and effective formats for presenting the information to maximize the benefits of such labeling. It is necessary to demonstrate that behavioral changes can occur by application of an SBDL.

However, application of a specific SBDL (month/day/year) at the manufacturer's level is a concept that has many practical limitations. The magnitude in number, diversity, and complexity of products that exist in the market place make practical implementation on a large scale of the Food Safety Objective (FSO)-based SBDL difficult. Accurate information on initial levels and growth rates of *L. monocytogenes* for many formulations are lacking, and a FSO tied to a public health goal has yet to be established.

Scientific parameters identified as important by the Committee include the following:

A. The pathogen of concern must be able to grow at refrigerated temperature in the food in question to a level that will be likely to cause illness in the host.
B. Scientific evidence that an SBDL will reduce the risk of foodborne illness for that food must be available.
C. Identification of safety-based end points is necessary for establishing an SBDL.
D. Determination of temperature to use for establishment of an SBDL.

The Committee determined that the following items need to be considered in the establishment of SBDLs:

- strain differences
- food matrices
- competing microflora and packaging
- production, distribution, and handling practices
- consumer susceptibility
- initial level
- growth kinetics

Verification and validation data necessary to demonstrate the effectiveness of an SBDL will differ depending on where the SBDL is applied. For example, at retail, a validated safe harbor may be used for an SBDL and verification could consist of assuring that the date is clearly visible, legible, and correctly applied. For manufacturers, use of an appropriate safe harbor value based on the literature, regulatory or industry guidelines, or other authoritative source; or generation of scientific data using modeling programs or laboratory experiments could be used for validation. The Committee developed guidance for conducting validation studies.

The Committee's hazard analysis led to the conclusion that the duration of refrigerated storage is not a major factor in foodborne illness caused by *Yersinia enterocolitica, Bacillus cereus,* and psychrotrophic *Clostridium botulinum*. Therefore, the Committee believes that an SBDL to limit the potential for growth of *L. monocytogenes* would have little or no impact on diseases related to these pathogens.

Educational efforts that focus on SBDLs should be combined with an educational effort that focuses on the importance of refrigeration temperature control. As consumers and food handlers increasingly appreciate the importance of adequate refrigeration, this should lead to a reduction in foodborne illness due to pathogen growth.

I. Introduction

There is a growing concern for the possible adverse impact of extended shelf life of certain foods on consumer health. The Food and Drug Administration (FDA) and Food Safety and Inspection Service (FSIS) draft and final risk assessments on *Listeria monocytogenes* in ready-to-eat (RTE) foods (*17, 18*) reinforced the critical interrelationship between the temperature and time of refrigerated storage on the microbiological safety of refrigerated RTE foods. In light of this, the National Advisory Committee on Microbiological Criteria for Foods (NACMCF) was asked by the supporting federal agencies to identify scientific parameters that should be considered for the establishment of Safety-Based "Use-By" Date Labels (SBDLs) and specify data needs for validating and verifying their adequacy.

The NACMCF approached this request by applying the procedures for a hazard analysis when developing a Hazard Analysis and Critical Control Point (HACCP) plan (*38*). The class of foods of concern was considered to be RTE foods that require refrigeration and support the growth of psychrotrophic pathogens. Four psychrotrophic pathogens warranted consideration: *L. monocytogenes,* non-proteolytic *C. botulinum, Y. enterocolitica,* and *B. cereus*. Upon further analysis, the Committee determined that *L. monocytogenes* is a significant hazard in this class of foods and an SBDL may be a useful control measure.

II. Purpose of the document

The purpose of this document is to address the following questions posed to the subcommittee:

1. What are the scientific parameters for establishing safety-based "use-by" date labels for refrigerated RTE foods?
2. What effect do the multiple factors that influence the growth and survival of *L. monocytogenes*, i.e., strain differences, food matrices, production and distribution systems, consumer susceptibility, etc., have on the establishment of safety-based "use-by" date labels for refrigerated RTE foods?
3. What data need to be acquired to scientifically validate and verify the adequacy of a proposed safety-based "use-by" date label for a refrigerated RTE food?
4. Should safety-based "use-by" dates for refrigerated RTE foods be established using mathematical modeling techniques? If so, what modeling approaches are best suited to the development of safety-based "use-by" date labels for refrigerated RTE foods?
5. What impact would safety-based "use-by" date labels, created for one psychrotrophic pathogen, e.g., *L. monocytogenes*, likely have on the control of other foodborne pathogens in refrigerated RTE foods?

To address these questions, the Committee defined the following terms:

A **ready-to-eat** (RTE) food is a food that is in edible form without additional preparation to achieve food safety (such as heating) but may receive additional preparation for palatability or aesthetic, epicurean, gastronomic, or culinary purposes.

A **safety-based date label** (SBDL) is labeling information regarding storage time to control the risk of illness from psychrotrophic pathogens. An SBDL may be a day/month/year or the number of days after purchase or opening and may include other statements such as "keep refrigerated" or "store below 40ºF."

A **Food Safety Objective (FSO)** is 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) (Codex Committee on Food Hygiene).

A **Performance Objective (PO)** is the maximum frequency and/or concentration of a hazard in a food at a specified step in the food chain before the time of consumption that provides or contributes to an FSO or ALOP, as applicable (Codex Committee on Food Hygiene).

A **Performance Criterion (PC)** is the effect in frequency and/or concentration of a hazard in a food that must be achieved by the application of one or more control measures to provide or contribute to a PO or an FSO (Codex Committee on Food Hygiene).

A **Psychrotroph** is an organism that grows well at or below 7°C and has an optimum growth temperature between 20° and 30°C.

A **Safe Harbor**, for the purpose of this document, is defined as a recognized procedure that can be employed without further validation studies.

The foods of concern in this document are refrigerated RTE foods for which the risk of disease may increase as a result of the growth of a psychrotrophic foodborne pathogen during adequate refrigerated storage and where date labeling would provide information that can be used to limit the extent of growth. The intent of an SBDL is to inform consumers and food handlers of the need to use the food within a certain time period. Controlled storage time can reduce the potential for development of high populations of psychrotrophic pathogens that could serve as a source of cross-contamination**.** It is important to note that an SBDL will not prevent illness if the food is heavily contaminated, held at high temperatures, or otherwise abused.

III. History

Manufacturers have rarely used date labeling to manage the safety of refrigerated RTE foods. Generally, manufacturers have applied date labeling to RTE products to reflect

the time period that the product retains best quality. Date labeling also has been useful for inventory control and traceability throughout the food chain. Several forms of date labeling have been used, and many are specific to the company manufacturing the food. "Closed date" coding involves the use of symbols or numbers that are not clearly recognized by consumers. The most easily recognizable form is "open dating," which involves a clearly distinguishable date code in a month, day, and year format. These are in a consumer recognizable format. These date codes are often prefaced by a statement such as: "Best if used by," "Sell By," "Use by," "Best if purchased by," or "Consume by."

Data collected by the Food Marketing Institute (FMI) indicate that consumers' perceptions vary regarding interpreting the dating statements used. According to the report, "consumers increasingly view spoilage as the predominant threat when asked to volunteer food safety concerns." Furthermore, the report states that "…foods not being fresh or past the expiration dates is the most frequently mentioned threat" (*19*). In a survey conducted in 2002, FMI reported that 54% of consumers believed that eating food past its sell-by/use-by date constituted a health risk (*20*). FMI concluded that if codes are to be reflective of product safety, consumer education will be needed in order for consumers to use the codes effectively (FMI presentation to this subcommittee). A consumer survey conducted for FSIS by RTI International reached a similar conclusion. This study found that "Some participants correctly define the different open date statements, while others find the use of different date statements confusing" (*5*).

The consideration of labels associated with refrigerated foods is not a new issue for the NACMCF. In 1990, the Committee was asked to provide recommendations for enhancing the microbiological safety of "refrigerated foods containing cooked, uncured meat or poultry products that are packaged for extended shelf life and that are RTE or prepared with little or no additional heat treatment." In its report, the NACMCF noted that:

> "Evidence suggests that consumers have difficulty distinguishing the differences between various food label instructions and their relationship to product safety. For that reason, and because of the greater temperature sensitivity of these products, the Committee recommends that retail and consumer packages carry a uniform standardized label statement and corresponding logo. More specifically, it is recommended that the following label be used on packaged foods that pose a safety hazard when subject to temperature abuse" (*36*).

*** IMPORTANT ***
MUST BE KEPT
REFRIGERATED

The National Conference on Weights and Measures developed a model "Uniform Open Dating Regulation" for consideration as a means of assisting state regulatory agencies in

addressing date labeling issues. The National Institute of Standards and Technology published the model regulation as Appendix A of Handbook #130 (39). The purpose of the model regulation, as stated in section 1.1 of the document is:

> "…to prescribe mandatory uniform date labeling of prepackaged, perishable foods and to prescribe optional uniform date labeling that must be used whenever a packager elects to use date labeling on prepackaged foods that are not perishable. Open dating is intended for use and understanding by both distributors and consumers when judging food qualities."

It is also interesting to note that in Section 3.3.1 "Sell By" dates are to be determined on the following criteria:

> "…allows a reasonable period after sale for consumption of the food without physical spoilage, loss of value, or loss of palatability. A reasonable period for consumption shall consist of at least one-third of the approximate total shelf life of the perishable food."

Certain states, as part of their labeling requirements, have adopted this regulation. Regulatory requirements relative to labeling within each state in the U.S. have been summarized (*32*). Currently, no state has a labeling requirement linked to food safety.

Although not required to do so, some companies have a long history of applying protocols to establish dating labels for selected products using scientific methods. The storage time and temperature expectations developed have been aimed at assuring consumer safety and product quality over the shelf life of the product. In such cases, the company may commission microbiological challenge studies, growth modeling, or both to determine potential growth of microbial pathogens. Such studies are laborious, time-consuming and resource-intensive to conduct and, thus are typically employed by companies with the financial and technical resources to use these techniques. Industry trade associations have also provided technical support for determining safety parameters.

While most date labeling practices and requirements are linked to quality factors, there are categories where labeling is required for nutritional adequacy or safety. For example, Federal regulations require a use-by date on the product label of infant formula under FDA inspection (21 CFR § 107.20(c)). This requirement is linked to nutritional adequacy; the assessment is based on the degradation of nutrients. An example of labeling requirements linked to safety is contained in the FDA 2001 Food Code. Section 3-501.17 of the model code addresses "Ready-To-Eat, Potentially Hazardous Food, Date Marking." This section imposes a commercial requirement of a maximum of seven days retail storage at 41°F or four days at 45°F for ready-to-eat potentially hazardous foods. "The date marking requirements apply to containers of processed food that have been opened and food prepared by a food establishment, in both cases if held for more than 24 hours, and while the food is under the control of the food establishment. This provision

applies to both bulk and display containers. It is not the intent of the Food Code to require date marking on the labels of consumer size packages" (*15*).

In a statement provided by FDA (December 15, 1999), the Agency notes that:

> "The dating provision was introduced into the Food Code because of the potential for growth over time of psychrotrophic organisms such as *Listeria monocytogenes* and *Yersinia enterocolitica* in refrigerated, ready-to-eat foods. Refrigeration does not prevent growth of these organisms. However, the rate of growth is slowed as the temperature decreases. Dating requirements are set forth to minimize a potential hazard attributable to the growth of psychrotrophic organisms during extended periods of refrigeration (*14*)."

The criteria for making the specific recommendations are described in the 2001 Food Code. The recommendation "…addresses time, in addition to temperature, as a control for the growth of *Listeria monocytogenes*, in refrigerated, ready-to-eat, potentially hazardous food. The Code provisions for cold holding focus on environmental conditions that allow 1 log of growth of *Listeria monocytogenes*, and does not set an acceptable number of *L. monocytogenes* in food" (*16*). The Food Code permits a variety of date coding practices (calendar dates, days of the week, color codes) but these codes are not intended for consumer packages.

IV. Hazard analysis

The Committee conducted a hazard analysis to identify organisms of concern for refrigerated RTE foods. Only those pathogens that can grow under refrigeration can feasibly be controlled by an SBDL. Therefore, the Committee identified four psychrotrophic pathogens for consideration: *L. monocytogenes*, non-proteolytic *C. botulinum*, *Y. enterocolitica,* and *B. cereus*.

The Committee considered epidemiological data in assessing the significance of these organisms in causing outbreaks associated with refrigerated RTE foods. While epidemiological data collected by state and local public health agencies in the course of outbreak and case investigations are a valuable source of information, the completeness of these data is limited by several factors. Clinicians typically do not request laboratory testing for self-limiting gastrointestinal illnesses (e.g., *B. cereus*) or spontaneous abortion (e.g., *L. monocytogenes*). Listeriosis that results in less severe manifestations than meningitis or death often does not receive laboratory investigation. Under-diagnosis is further complicated by typical cultural procedures employed in clinical laboratories. Routine stool culture procedures do not include media for the isolation of *B. cereus* or *Y. enterocolitica.* The identification of these organisms would only be possible if there were a specific request for these pathogens.

Listeria monocytogenes

Data from active surveillance in selected sites indicate that the annualized incidence of culture-confirmed *L. monocytogenes* infections decreased from 7.9 per million persons in 1989 to 4.4 per million in 1993. Preliminary data from the Foodborne Diseases Active Surveillance Network (FoodNet) indicate a rate of 3.3 cases per million in 2003 (*8*).

Between 1973 and 2000, the Centers for Disease Control and Prevention (CDC) received reports of 14 foodborne outbreaks due to *L. monocytogenes* infections. These accounted for 487 illnesses, 235 hospitalizations, and 111 fatalities, including miscarriages and stillbirths (P. Griffin, personal communication). Specific food products were implicated in nine outbreaks, suspected in four outbreaks, and unknown in one. The implicated products were refrigerated RTE foods and included milk (2 outbreaks), Mexican-style cheese (2 outbreaks), hot dogs (2 outbreaks), deli meats (2 outbreaks), and pâté (1 outbreak); the suspected products were raw vegetables, shrimp, deli meats, and hot dogs.

L. monocytogenes has been found in a wide variety of raw agricultural commodities of animal and plant origin (*37*). In manufacturing, the persistence of *L. monocytogenes* in the environment has been clearly demonstrated (*44*). While manufacturers utilize control measures to reduce or eliminate the level of *L. monocytogenes*, a small percentage of products may still be contaminated.

Environmental contamination has also been demonstrated at the retail level. Meat slicing equipment has been reported to harbor *L. monocytogenes* (*29*). There is an opportunity for cross-contamination when food handlers do not follow proper hygienic practices, which can also happen in the home. The CDC demonstrated *L. monocytogenes* cross-contamination of foods within the refrigerators of patients with listeriosis (*42*).

The 2003 FDA/FSIS *L. monocytogenes* risk assessment predicts that increased storage temperature and time for refrigerated foods are associated with increased mortality in the elderly population (*18*). The Committee concluded that *L. monocytogenes* is a significant hazard when present in refrigerated RTE foods that support growth and an SBDL may be a useful control measure that has potential value for reducing consumer risk, particularly when combined with improved temperature control.

Yersinia enterocolitica

Between 1973 and 2000, CDC received reports of ten foodborne outbreaks due to *Y. enterocolitica* infections. Among the eight outbreaks with a known food vehicle, four were due to a refrigerated RTE food (P. Griffin, personal communication).

Most strains of *Y. enterocolitica* found in foods are non-pathogenic (*30*). Swine serve as the natural ecological niche of pathogenic biotypes. Cross-contamination of RTE foods from raw pork has been a source of yersiniosis. Four of the eight outbreaks with a known vehicle were due to raw agricultural products (bean sprouts, vegetables, pork chitterlings (2)). Based on the Committee's knowledge of U.S. outbreaks since 1973, it concluded

that none of the outbreaks associated with refrigerated RTE foods would have been prevented by an SBDL because time under adequate refrigeration conditions was not a contributing factor.

Psychrotrophic *Clostridium botulinum*

Among psychrotrophic strains of *C. botulinum*, only toxigenic type E strains have caused outbreaks in the United States. Between 1973 and 2000, CDC collected data on over 150 patients with botulism due to botulinum toxin type E in Alaska. All with a known food vehicle were due to fish or aquatic mammal products (e.g., fermented fish heads, fermented beaver tail). None of the outbreaks were from a commercial source. Between 1990 and 2000, CDC collected data on 24 outbreaks of botulism due to botulinum toxin type E in the United States, of which all except two were in Alaska. All were from fish or marine mammal products; only one was identified as being from a commercial source. A 1989 CDC analysis of U.S. outbreaks indicated that no cases of botulism due to non-proteolytic strains were obviously due solely to growth at refrigeration temperatures (*27*). This epidemiological picture has not changed in the years since. Therefore, the Committee concludes that an SBDL would have little impact on preventing outbreaks associated with psychrotrophic *C. botulinum.*

Bacillus cereus

B. cereus is present in a wide variety of foods. Certain strains of *B. cereus* are capable of growth under refrigeration conditions (*25*). According to the International Commission on Microbiological Specifications for Foods (ICMSF), "Every well-documented report of *B. cereus* intoxication has described time/temperature abuse that has enabled relatively low (innocuous) levels of *B. cereus* in foods greatly to increase" (*30*). Due to the less severe symptoms caused by this organism, epidemiological data are more subject to reporting error than pathogens such as *L. monocytogenes*. There is insufficient information on the potential for toxin production and/or growth to high numbers that could lead to illness in adequately refrigerated foods. Despite microbiological data suggesting that *B. cereus* can grow in refrigerated RTE foods, adequate refrigeration appears to provide appropriate control; this suggests that SBDLs may have little impact on preventing illness from *B. cereus*.

V. Safety-Based Consume-by Date Labels Approaches and Formats

More than one format can be used for an SBDL, e.g., 1) an open code format of a specific day/month/year; 2) a specific time that begins after purchase by the consumer or after the package has been opened; or 3) a combination of the two. Selection of format for SBDLs should consider various factors, including the potential sources of contamination, the likelihood of recontamination, and handling and use through manufacturing, distribution, sale, and consumer storage.

There are several points in the food chain (manufacturing, retail, food service establishments, and the home) where contamination by *L. monocytogenes* can occur. Therefore, SBDLs may be applied at multiple points in the food chain. While date labels may be determined and applied by the manufacturer for some products, there are some instances where the SBDL would be better determined and applied elsewhere in the food chain. Selected examples are described below.

Example 1: Cook-in-bag meat products are processed to destroy *L. monocytogenes.* They are stored and distributed under controlled temperatures, including freezing. Once the bag is opened, it is exposed to potential contamination at retail or in the home. A retailer could slice and package the product and apply a "Consume-by" date label using the information provided by the supplier. Consumers would benefit from a "Use within *x* days of opening" statement on the package.

Example 2: A retailer may receive frozen cooked RTE chicken and use this in preparation of a salad. It would be the retailer's responsibility to establish an SBDL or use a safe harbor appropriate for this product, and the consumer would benefit from a "Consume by" date.

Example 3: A manufacturer may set a "Use-by" date on packages of luncheon meat. Because consumers may contaminate the product after opening, the manufacturer may also include a "Consume within *x* days of opening" for products that support growth.

VI. Questions & Answers

The Committee was asked to answer five questions. These were answered using *L. monocytogenes* as the organism of concern based on the results of the previously described hazard analysis. However, the principles could apply to other pathogens of concern.

1. What are the scientific parameters for establishing safety-based "use-by" date labels for refrigerated RTE foods?

The first step in establishing an SBDL is to conduct a hazard analysis to identify the pathogen(s) of concern for the food that would be impacted by an SBDL. Once the pathogen of concern has been identified (in this case, *L. monocytogenes*) to be a hazard reasonably likely to occur, the following scientific parameters should be considered.

A. The pathogen of concern must be able to grow at refrigerated temperature in the food in question to a level that will be likely to cause illness in the host.

B. Scientific evidence that an SBDL will reduce the risk of foodborne illness for that food must be available.

C. Identification of safety-based end points and SBDLs are necessary.

D. The temperature to use for the establishment of an SBDL must be determined.

To establish the above parameters, the following information is also needed and is discussed under Question 2:

- Strain differences
- Food matrices, competing microflora, and packaging
- Production, distribution, and handling
- Consumer susceptibility
- Initial level
- Growth kinetics

A. **The pathogen of concern must be able to grow at refrigerated temperatures in the food in question to a level that will be likely to cause illness in the host.**

The utility of an SBDL is based on the premise that increased numbers of organisms increase the risk presented by the food. Without the opportunity for *L. monocytogenes* numbers to increase in a food, the expected level of contamination on the food would have a relatively low probability of causing listeriosis. The FDA/FSIS risk assessment has shown that the ability of the food to support growth is a critical factor (*18*). If the food can support growth, then sufficient time and temperature are necessary for the growth to occur. Of the two, temperature has a greater impact on the amount of growth; however, allowing for the variation in distribution of contamination, growth rates, and temperatures, limiting the excessive storage times will reduce the incidence of listeriosis. If limiting storage times is combined with temperature control, the incidence of listeriosis would be greatly reduced. (See Figure 1.)

B. **Scientific evidence that an SBDL will reduce the risk of foodborne illness for that food must be available.**

The FDA/FSIS risk assessment scenarios also show that a reasonable or feasible storage time limitation cannot completely overcome the impact of excessive storage temperatures or contamination (*18*). The storage time limitation should be viewed as an additional control to supplement good manufacturing practices and adequate refrigeration temperatures. To have an SBDL with feasible storage periods for the U.S. food distribution system and consumer expectations, the temperature used to determine the SBDL will need to be set using temperatures approximating conditions the food will normally encounter after final packaging.

As an example, the interaction of time and temperature in the home on the predicted annual mortality rate in the elderly subpopulation attributed to listeriosis from deli meats (non-fermented luncheon meats purchased as pre-packaged or deli-sliced) was simulated (*18*). The baseline model (maximum 28 days of storage) estimated 228 deaths in the elderly from deli meats, as shown in Figure 1. Each line represents a maximum storage time over the range of maximum refrigerator temperatures. Eliminating storage temperatures above approximately 8°C or all storage times longer than approximately 8 days would achieve a 50% reduction in mortality from listeriosis. If all temperatures were less than 6°C (43°F), predicted mortality would be very low and little difference in mortality would be predicted for deli meat stored for up to 28 days (the baseline). Thus, for refrigerators operating at temperatures above 6°C (43°F), longer shelf life results in an increased risk of mortality as temperature increases.

For other food groups, risk will vary depending on how contamination levels, growth rates, temperatures, and times interact. However, the scenarios clearly show that without the opportunity for *L. monocytogenes* numbers to increase in a food, the food would have a relatively low probability of causing listeriosis.

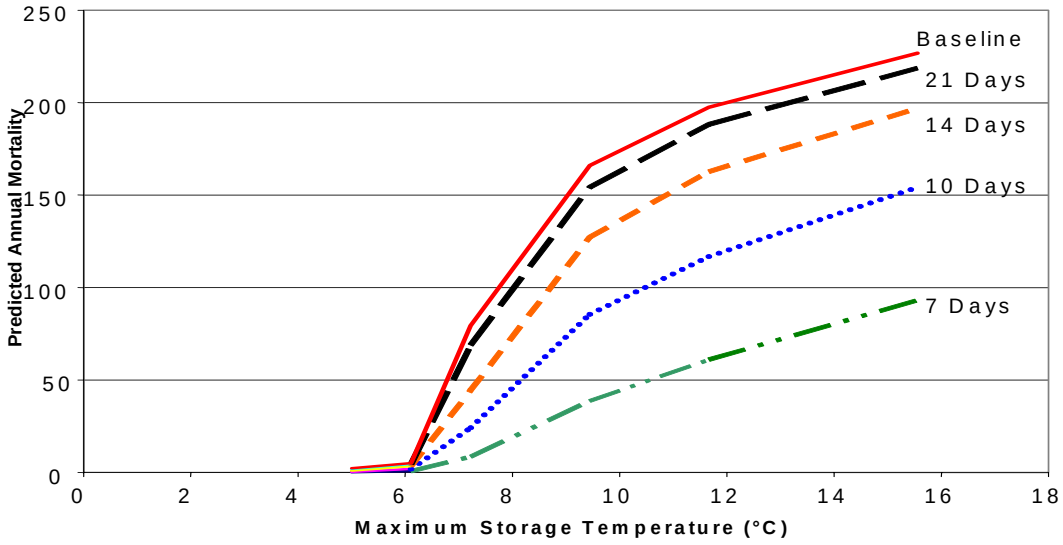

Figure 1. Predicted annual mortality in the elderly subpopulation attributible to deli meats as a function of maximum storage time and maximum storage temperature

C. Identification of safety-based endpoints is necessary for establishing an SBDL.

At least two approaches can be used to establish an endpoint for an SBDL.

- One approach is to set the acceptable level at the point of consumption (*i.e.*, an FSO). This involves specifying the maximum tolerable level, estimating the initial level, and determining the time to reach the maximum level. This is the scientifically preferred approach because it can be linked to a public health objective and provides a similar level of risk for all foods. The limitation to this approach is that the maximum tolerable level (the FSO) must be articulated by an authority such as a federal agency. A second limitation is that it may be difficult to establish an initial level, particularly when the frequency and concentration is consistently below the level of detection. For certain foods this approach could be used by a manufacturer to arrive at an SBDL formatted as month/day/year. The same foods also may have a label statement that specifies when a food must be used, for example, after opening or purchasing from a deli counter.

- Another approach is to use a performance criterion in which a maximum increase in the number of organisms is specified. This approach does not require knowing the initial level of contamination, which is useful when initial levels are difficult to estimate (e.g., cross-contamination). However, this approach does provide varying levels of risk and protection depending on the initial pathogen concentration. This approach can be useful for establishing storage time after opening by the consumer or food handler (e.g., "use by *x* date," "use within *x* days of opening"), and may be suitable for setting a safe harbor.

D. Determination of temperature for establishment of an SBDL

Foods are exposed to a wide variety of time/temperature combinations from farm, manufacturing, distribution, display, transport, preparation, storage, and use. The SBDL will need to be set using temperatures approximating conditions the food will normally encounter after final packaging. For most manufactured refrigerated RTE foods, there is good temperature control from packaging through distribution to retail. FSIS requires that meat and poultry products be shipped at temperatures not exceeding 40°F. The 2001 Food Code has established 41°F as the appropriate temperature for storage of potentially hazardous foods. However, Audits International reported that 90% of home refrigerators in the U.S. were below 45°F (*1*). The Committee agreed that 45°F could represent a standardized refrigeration temperature to reflect the consumer segment of refrigeration storage (e.g., for determination of a "consume within *x* days after opening").

The Committee agrees with the 2001 Food Code that ≤ 41°F is optimal. However, the Committee recognizes that many consumer refrigerators are ≤ 45°F. Therefore, 45°F should be used for establishing an SBDL for the period of time that would reflect consumer handling.

The Committee recognizes that foods actually encounter a range of temperatures below and above 45°F. Lower temperatures are likely encountered in processors' warehouses and distribution centers. Higher temperatures are likely encountered in grocery store deli cases, during transport in consumers' cars, and while foods are being served (*1*).

2. What effect do the multiple factors that influence the growth and survival of *L. monocytogenes*, i.e., strain differences, food matrices, production and distribution systems, consumer susceptibility, etc., have on the establishment of safety-based "use-by" date labels for refrigerated RTE foods?

As previously mentioned, the extent of growth influences the likelihood of causing listeriosis. A number of factors could have a significant effect on the growth of *L. monocytogenes* under refrigeration and hence impact the setting of an SBDL. One must consider how each product and factor interacts to limit growth of the pathogen of concern.

A. Strain Differences

Different strains have different growth rates; therefore, challenge studies to establish SBDLs must reflect this variability (*2, 3, 11, 49*). Generally, a mixture of three to five strains is used to determine growth or survival so that diversity is considered (see Appendix I - Guidance for conducting microbial challenge studies of refrigerated RTE foods to validate an SBDL).

B. Food Matrices, Competing Microflora and Packaging

The nature of the food matrix is an important factor in the growth and survival of *L. monocytogenes*. Some food matrices support rapid growth while others are inhibitory. If the food does not permit growth of *L. monocytogenes*, then an SBDL is not warranted. Risk to all susceptible consumers may be reduced by reformulating RTE foods to inhibit growth of *L. monocytogenes*. SBDLs of foods that completely or substantially inhibit the growth of psychrotrophic pathogens would not have a significant impact on the number of cases of illness because the pathogen numbers would not increase with increasing storage to populations likely to cause illness. In many of these inhibitory foods, pathogen numbers actually decrease during storage making them, paradoxically, safer with extended storage, e.g. yogurt.

Intrinsic characteristics, such as water activity (a_w), reduction-oxidation potential, pH, salt content, moisture level, and natural and added inhibitors, interact to influence the growth and survival of microorganisms. A review of these factors can be found in Institute of Food Technologists (IFT)/FDA Task Order 4 on Potentially Hazardous Foods (*31*). When designing growth and survival studies, it is necessary to understand the role of these factors in the food under consideration and account for variations that exist.

The impact of competing microflora in a specific food type should be taken into account in predicting the potential growth of the pathogen. The competing microflora can impact the growth kinetics of *L. monocytogenes.* Challenge studies have demonstrated that *L. monocytogenes* can sometimes grow to high numbers on foods in the presence of a product's normal microflora, especially when inoculated at artificially high levels. In other instances, competing flora can result in spoilage before *L. monocytogenes* can grow to high numbers. When both spoilage flora and pathogen levels are low, competition

probably does not affect the growth of either. However, when the spoilage flora reach high levels (ca. > 10^6/g), growth of all microorganisms, including *L. monocytogenes,* reach a plateau. This phenomenon suggests that in RTE foods, *L. monocytogenes* growth may not routinely reach the levels indicated by the challenge studies.

Modern food packaging, which has been developed to inhibit spoilage of foods, can have a significant impact on the growth and survival of *L. monocytogenes*. For example, certain modified atmospheres can inhibit or delay the growth of *L. monocytogenes* in products such as cottage cheese packaged with dissolved CO_2 (*10*). Conversely, as shelf life is increased due to inhibition of competing flora, the risk of growth of *L. monocytogenes*, if present, may increase in certain packaged foods during extended consumer storage.

C. Production, distribution and handling

Environmental contamination of product during manufacture influences the initial level of *L. monocytogenes.* For products that support growth, temperature controls during production and distribution are important to ensure that temperatures remain within the range used to set the SBDL, thereby limiting growth. Temperatures of foods are generally well-controlled during production and storage by the manufacturer, however, temperature control is more variable during retail display and consumer storage (*1*).

Refrigerated RTE foods are produced, distributed, stored, and displayed using a wide variety of procedures. Each pathway has unique considerations with respect to the potential for contamination with and subsequent growth of *L. monocytogenes* and hence for establishment of an SBDL. It may be necessary to apply SBDLs at different points in the food chain. Consideration should be given to the following:

Refrigerated distribution: The most extensive of the product classes are those processed, packaged, stored, transported, and displayed, and then stored again under refrigerated conditions by the consumer. Examples of this type of food are pre-packaged RTE luncheon meats, frankfurters, milk, fresh-cut fruits and vegetables, soft cheeses, and hot smoked fish.

Frozen distribution and slacking out: Products are processed and frozen before or after packaging, and then distributed in a frozen state. The product is then thawed (slacked out) for display at retail and subsequently stored at refrigerated temperatures by the consumer. Thus, refrigerated storage would begin from the period of thawing at the retail establishment. Examples of this class of products are RTE shrimp, some smoked salmon, and some prepared entrees.

Packaging at retail: Products are prepared and packaged in one facility and distributed in bulk to the retail market where they are sliced/portioned/packaged for display or upon demand. Examples of this class of products are RTE deli meats sliced at retail, meat and seafood salads, and fresh produce that are sliced or portioned at the retail establishment.

Foods in the home: Some products are treated to destroy psychrotrophic pathogens in aseptic systems or after packaging at the manufacturer. The potential for contamination therefore occurs when the packages are first opened by the consumer. The likelihood of contamination depends on the level of control practiced by the food handler. Examples of this type of food include pre-packaged RTE luncheon meats and hot dogs. Foods cooked by consumers and stored as leftovers can also support the growth of psychrotrophic pathogens and would fall in this category.

Single-use versus multiple-use: The extent and nature of handling that a product receives after the package is opened may influence the risk of contamination. For example, food in a single-use container consumed immediately after opening with little potential for recontamination. Conversely, a bulk package containing many slices (luncheon meats) or servings (milk) that is used over a period of time is potentially subject to recontamination and subsequent growth.

D. Consumer susceptibility

Risk assessments have evaluated relative susceptibility to listeriosis for varying subpopulations (*13, 18*). SBDLs should consider that healthy and vulnerable populations, including the elderly and pregnant women, will consume targeted products. Vulnerable populations, *e.g.*, immunosuppressed, should take extra precautions and should also rely on health care provider information and targeted consumer education to limit the risk of listeriosis.

E. Initial level

The initial level of *L. monocytogenes* in a food is an important factor in determining the SBDL when using an end point based on an FSO. Recent quantitative surveys of RTE foods in the U.S. marketplace have demonstrated that a small percentage of retail foods contain *L. monocytogenes* (*18, 21, 23*). The rates of contamination range from less than 0.1% in pasteurized milk (*21*) to 4.7% for seafood salads (*23*). When foods in these surveys contained *L. monocytogenes*, the levels were usually low (< 1 cfu/g). However, some consumer packages had higher levels at retail, including more than 1,000 cfu/g. Table 1 presents data taken from these studies.

Table 1. Contamination at retail of refrigerated RTE foods with *L. monocytogenes*

Food Category	Number of samples with different contamination levels (cfu/g)									
	Total number	< 0.04[a]	To 0.1	0.11 - 1	1.1 - 10	11 - 100	101 – 1,000	1,001 – 10,000	To 100,000	To 1,000,000
Smoked Seafood	2644	2530	67	11	19	8	6	1	0	2
Bagged Precut Leafy Salad	2966	2944	17	1	1	2	1	0	0	0
Fresh Soft Cheese	2931	2926	2	0	0	3	0	0	0	0
Soft, Mold-ripened Cheese	1347	1333	12	0	2	0	0	0	0	0
Blue-veined Cheese	1623	1600	18	3	1	1	0	0	0	0
Pasteurized Milk[b]	5804	5803	1	0	0	0	0	0	0	0
Deli Meats	9199	9117	42	20	10	2	7	1	0	0
Deli Salads	8549	8347	162	28	9	2	0	1	0	0
Seafood Salads	2446	2331	82	19	10	2	2	0	0	0

[a] < 0.04 cfu/g indicates the samples had undetectable levels of *L. monocytogenes*
[b] data provided by International Dairy Foods Association *(21)*

A recent study of frankfurter production lots demonstrated the sporadic nature of *L. monocytogenes* contamination (*45*). An entire lot was obtained from each of 12 plants and approximately 2700 samples were taken from each lot (32,800 total samples). Using a package rinse sampling protocol, at least one positive sample was obtained from 7 of the 12 lots (58% positive). In four of these positive lot plants, no more than four positive samples were found. The remaining three plants had 44 (1.5%), 51 (2.2%) and 437 (15.6%) positive samples. Overall 1.65% of the samples were positive. Except for the plant with the highest prevalence, *L. monocytogenes* was infrequently present.

Cross-contamination has been recognized as an additional potential route of contamination for many pathogen-food pairs. A home refrigerator study of listeriosis cases found that *L. monocytogenes* could be isolated from at least 1 food item in 64% of the refrigerators (*42*). *L. monocytogenes* was found in 7.6% of RTE samples, including processed meats, leftovers, cheeses and raw vegetables. The frequencies of contamination suggest cross-contamination in the home. Studies of consumer food handling knowledge and practices also reveal the likelihood of widespread cross-contamination and inadequate hand washing (*9, 22, 24, 26, 34, 48*). Various bacterial cross-contamination rates were determined by Chen *et al.* (*9*). The ranges in the percentages of bacteria transferred from chicken meat to hands was 0.2% to 8.7% and from cutting boards to lettuce was 0.2% to 7.9%, for example. Cross-contamination can also occur in retail delicatessen and other food service/sales facilities where foods are handled and repackaged.

These cross-contamination findings imply that *L. monocytogenes* or other microbial pathogens could be transferred to a food that is more favorable for growth, stored for

longer times, or stored at higher temperatures than the originally contaminated food. This particularly applies to foods that are usually processed and packaged so they are free of *L. monocytogenes* at purchase, but are packaged in multiple-serving containers that will be repeatedly opened, have servings removed, and be returned to refrigerated storage.

F. Growth kinetics

For refrigerated food products that support the growth of *L. monocytogenes*, the number of pathogens after a period of storage is a function of the initial number of pathogens, the temperature and time at which the product is stored, the type and number of competitive microflora, and intrinsic properties of the product.

Figure 2 illustrates an example from the USDA Pathogen Modeling Program where *L. monocytogenes* at 5° and 7°C in broth medium has growth rates of 0.77 and 1.12 logs/ day, respectively. The survey of the literature in the FDA/FSIS 2003 risk assessment found average growth rates (standardized to 5ºC) for various categories of foods to be 0.38 log/day for cooked RTE crustaceans, 0.28 log/day for deli meats, 0.26 log/day for milk, 0.25 log/day for pâté and meat spreads, and 0.15 log/day for smoked seafoods, for example (*18*). Based on this, the broth model estimates a faster growth rate than the average reported in the literature.

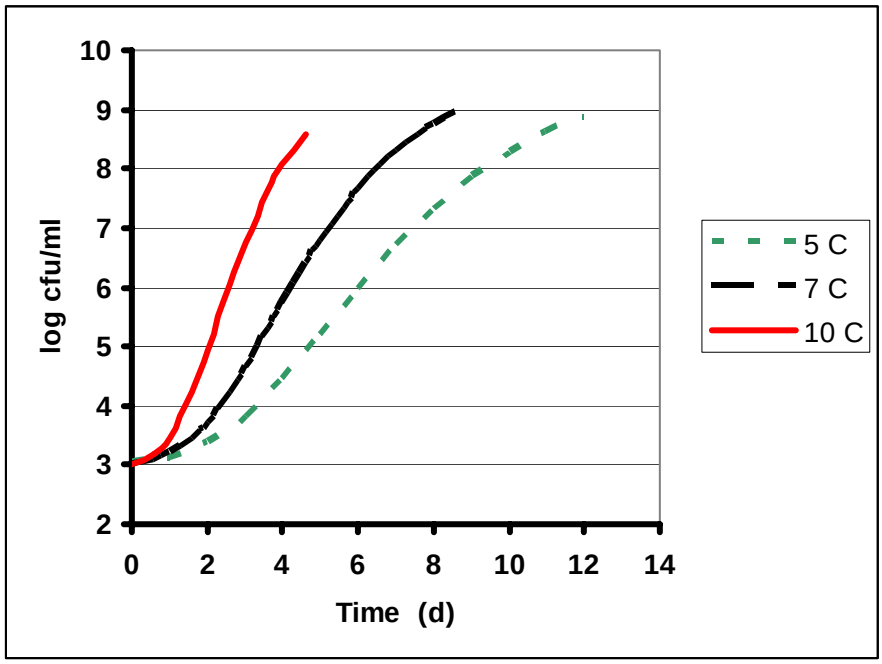

Figure 2. Growth of *L. monocytogenes* at 5, 7, and 10°C (data from USDA Pathogen Modeling Program version 7.0, aerobic growth, pH 6.8, 0.8% NaCl, a_w 0.996)

The shelf life is a function of both growth rate and lag time. Lag time is the time required for an organism to adjust its composition and metabolism to the new conditions and initiate multiplication. The length of time for the cells to do this is highly dependent on temperature. For example, the lag time for growth of *L. monocytogenes* at 10°C (50°F) is 1.5 days, while at 1°C (34°F) lag time is approximately 3.3 days (*35*). Likewise, at 10°C (50°F), the generation time for the same organism is 5-8 h, while at 1°C (34°F), the generation time is between 62 and 131 h.

Figures 3 and 4 show the effect of temperature and pH on lag times of *L. monocytogenes* cells grown at optimal conditions to the stationary phase. Using the USDA Pathogen Modeling Program (version 7.0), it was determined that lag times of stationary phase *L. monocytogenes* in broth medium at 5 and 10°C were 140.5 and 62.6 h, respectively (based on aerobic growth, pH 5.5, and 2% NaCl). However, lag phase duration is dependent on the physiological state of the cells, their original temperature and medium, and their new temperature and medium (*49*). Cells experiencing a temperature decline have lengthy lag times compared to cells experiencing no temperature change or a temperature increase. Cells in the exponential growth phase have relatively brief lag times, stationary and starved cells have longer times; frozen cells and desiccated cells have lengthy lag times. These times reflect the periods necessary for cellular adjustments and perhaps for repairs necessary for growth to resume.

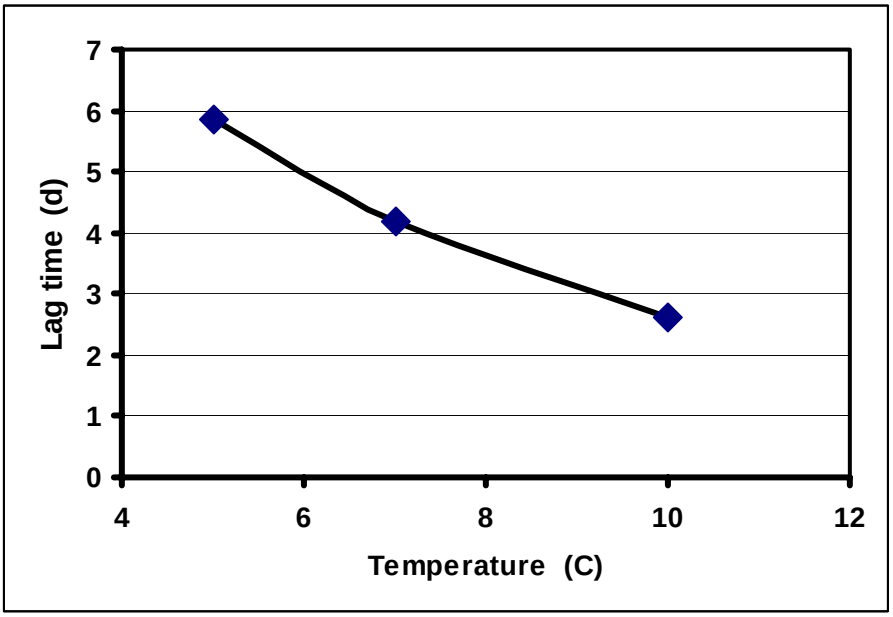

Figure 3. Effect of temperature on lag times for *L. monocytogenes* (data from USDA Pathogen Modeling Program version 7.0, aerobic growth, pH 5.5, 2% NaCl, a_w 0.989)

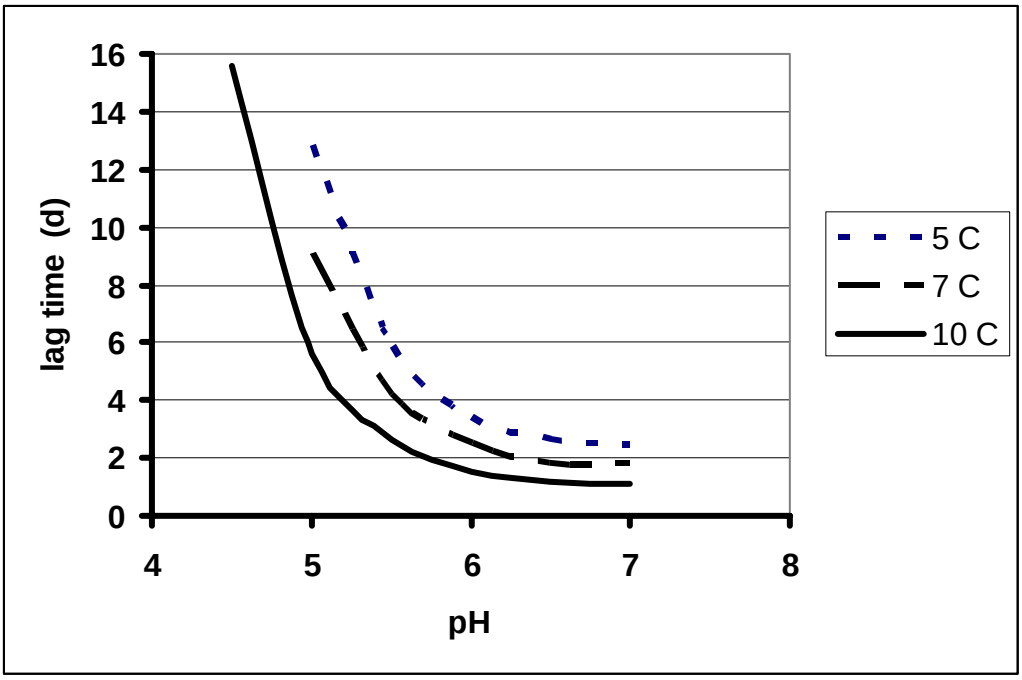

Figure 4. Effect of pH on lag times for *L. monocytogenes* (data from USDA Pathogen Modeling Program version 7.0, aerobic growth, 2% NaCl, a_w 0.989)

L. monocytogenes is capable of growth to high levels in high-moisture, neutral-pH foods (e.g., milk, cooked turkey meat, pâté, and Mexican-style soft cheese) during refrigerated storage. Duffes *et al.* (*12*) showed maximum levels (cfu/g) in smoked salmon to reach $10^{4.8}$ at 4ºC and $10^{8.1}$ at 8ºC. Pelroy *et al*. (*41*) found maximum levels in smoked salmon to be 10^5 and $10^{6.5}$ cfu/g at 5 and 10ºC, respectively. Maximum populations reported in cream were 10^7 and $10^{7.5}$ cfu/g at 4 and 8ºC, respectively (*43*); in butter it was reported as $10^{5.5}$ and 10^6 cfu/g at 4 – 6ºC and at 13ºC, respectively (*40*); and in lettuce it was reported as 10^5 to $10^{5.5}$ cfu/g at 5ºC and $10^{6.5}$ to $10^{7.5}$ at 10ºC (*4*).

3. What data need to be acquired to scientifically validate and verify the adequacy of a proposed SBDL for a refrigerated RTE food?

Ultimately, the success (adequacy) of the SBDL concept depends on how the consumer interprets and uses the information measured both before and after implementation. The validity of this concept should be determined by the regulatory agency. Data should be collected about at-risk consumers' knowledge, attitudes, and practices in relation to SBDLs (refrigeration times and temperatures). Label wording and display, education, and consumer motivation need to be effectively used to maximize the benefits of labeling. It is necessary to affect more than the consumers' knowledge and awareness of an SBDL. To have an impact on public health, appropriate behavioral changes in actually following the SBDL must occur. Information may need to be targeted to specific populations that are at greater risk. Additionally, surveillance data on foodborne illness should be monitored to determine the success with respect to public health.

Focus groups can help determine consumer knowledge and effective formats for presenting the information. This may include standardized placement and wording of the SBDL on the food packages. It may also require effective public education to instruct and motivate consumers in using this information.

Verification and validation data will differ depending on where the SBDL is applied. For example, at retail, a safe harbor may be used for an SBDL and verification could consist of assuring that the date is clearly visible, legible, and correctly applied. For manufacturers, the following check list may be appropriate to scientifically validate and verify the adequacy of an SBDL.

A. Validation

1. Documentation to support that the hazard analysis has correctly identified the potential pathogens that could multiply during refrigerated distribution and storage;
2. Use of an appropriate safe harbor value based on the literature, regulatory, or industry guidelines, or other authoritative source; or generate data using modeling programs or laboratory experiments based on an acceptable challenge protocol such as that provided in this document (Appendix I) for establishing the SBDL;
3. Assurance that the parameters used in laboratory experiments, modeling, and other studies cover the breadth of product and packaging characteristics (e.g., pH, organic acid concentration, salt, nitrite concentration, and packaging conditions);
4. Assurance that the distribution conditions and storage temperatures used for predictive modeling or challenge tests to establish the SBDL are appropriate for the product.

Use of an expert to review the validation protocols, testing, and conclusions may lead to greater assurance that the validated SBDL is appropriately set and applied.

B. Verification

Implementation of an SBDL should follow recognized procedures for application of other important label information. Verifying that the SBDL is being applied correctly by an establishment (e.g., manufacturing plant, retail establishment, or restaurant) will require on-going activities that are beyond the scope of the question. However, once the use of an SBDL is implemented, the following could be components of verification:

1. Standard operating procedures that ensure that products for which an SBDL is necessary, have SBDLs that are, clearly visible, legibly and correctly applied;

2. Records showing that an appropriate SBDL has been applied to the product (e.g., code dating, production dating, lot numbers);
3. Records for reassessment of the SBDL when significant changes in product formulation, packaging, distribution, or storage have been implemented;
4. Records of laboratory analyses that verify that the microbial hazard of concern is absent or below a specified concentration on the SBDL under refrigerated storage (i.e., the temperature conditions used in the validation work to establish the SBDL).

4. Should safety-based "use-by" date labels for refrigerated RTE foods be established using mathematical modeling techniques? If so, what modeling approaches are best suited to the development of labels for refrigerated RTE foods?

During the past decade, there has been extensive research directed toward the development of mathematical models that describe the growth characteristics of *L. monocytogenes* and other foodborne pathogenic bacteria. Mathematical modeling techniques can be used to estimate the growth of *L. monocytogenes* in foods, but they should be used in conjunction with other information as needed. Considering the wide range of products, product formulations, and production facilities, as well as the wide diversity of practices associated with the distribution, marketing, and consumption of RTE foods, it does not seem feasible to conduct inoculated pack studies on more than a limited number of product classes and pathways. Accordingly, it is assumed that the modeling of microbial growth will play an important role in the development of SBDLs.

Software programs such as the USDA Pathogen Modeling Program (*45*) provide growth models for most foodborne pathogens in broth media. The impact of changes in temperature, pH, salt levels, and other factors can readily be determined. The UK Food MicroModel also has an extensive growth modeling capability. Many of these models are based on growth in inoculated foods. A joint USDA Agricultural Research Service—UK Institute of Food Research and the UK Food Standards Agency program, called ComBase (*46*), provides a computer searchable database that can quickly identify and present growth data from published literature that fit the specified search criteria.

Current growth rate models tend to be conservative with respect to most foods because the models have been developed using pure cultures in laboratory media. As discussed in the previous section on Growth kinetics, the Pathogen Modeling Program generally provides a conservative prediction, i.e., it usually over predicts the amount of growth, and can be used as a "safe harbor." Current growth models do not consider the prior history or physiological state of the contaminating cells.

The relevance and validity of a model must be carefully evaluated when determining the degree of confidence that can be given to a model's predictions. More rigorous verification may be needed where there is less confidence in the model. If the initial estimate clearly indicates that growth of *L. monocytogenes* is not likely to occur within the expected or desired shelf life of the product during normal distribution and storage

conditions (with a reasonable margin of safety), then further validation is not needed. If growth is possible, specific inoculated product trials may need to be conducted to determine actual growth rates at refrigeration temperatures for that food. Models can assess changes in formulation, contamination levels, storage times and temperatures, but should be backed by some links to challenge studies.

Combining separate models for pathogen growth and lag, adjusting them for competition from the spoilage flora, and determining the spoilage time is currently a highly uncertain process. Validation of the model in the food and under pilot plant conditions reduces the uncertainty compared to models based solely on pure cultures grown in broth. For models to have utility for small processors, they must be simple to use, contain appropriate parameters for the food, and be validated for the specific food product. Unless specific information or models have been developed for the food in question, models or other data should represent the most rapid growth of pathogens of concern for the conditions under consideration.

5. What impact would safety-based "use-by" date labels created for one psychrotrophic pathogen, e.g., *L. monocytogenes,* likely have on the control of other foodborne pathogens in refrigerated RTE foods?

The impact will depend on the specific food-pathogen combination. Based on the epidemiological information, the vast majority of cases of foodborne illness are due to pathogens that are incapable of growth in food at refrigeration temperatures (*33*). Thus an SBDL for refrigerated products would have no impact on foodborne illnesses caused by these pathogens.

For pathogens that can multiply in refrigerated RTE foods, the factors of primary concern are the likelihood of contamination with a particular psychrotrophic pathogen, the level of contamination, the rate of growth at refrigeration temperatures, storage and handling practices, and the level required to cause illness. The SBDL should be set for the pathogen identified through the hazard analysis that is most likely to first reach the level of public health concern under refrigeration. The Committee determined that this is *L. monocytogenes* for most refrigerated RTE foods. An SBDL for refrigerated RTE foods would only have an impact on organisms that can grow under refrigeration. The SBDL is a risk management strategy that would not likely be beneficial for other psychrotrophic pathogens. It would not be effective for preventing illness from pathogens such as *Salmonella*, *Escherichia coli* O157:H7, and viruses that survive but do not grow in foods at refrigeration temperatures. As noted in question 4 above, the effectiveness of an SBDL is greatly dependent on how the consumer and food handler interpret and react to the labeling.

Furthermore, the Committee's hazard analysis led to the conclusion that the duration of refrigerated storage is not a major factor in foodborne illness caused by *Y. enterocolitica, B. cereus*, and psychrotrophic *C. botulinum.* Therefore, the Committee believes that an

SBDL to limit the potential for growth of *L. monocytogenes* would have little or no impact on diseases related to these pathogens.

The FDA/FSIS risk assessment demonstrated that the impact of temperature on the risk of listeriosis was significantly greater than the impact of time. Educational efforts that focus on SBDLs should also emphasize the importance of refrigeration temperature control. As consumers and food handlers increasingly appreciate the importance of adequate refrigeration, this will lead to a reduction in foodborne illness due to pathogen growth.

VII. Conclusions

In the event an SBDL concept is pursued, *L. monocytogenes* has been identified as the appropriate target organism for refrigerated RTE foods that support its growth.

Given the morbidity and high mortality of *L. monocytogenes* infection and the association of *L. monocytogenes* with refrigerated foods, the Committee believes the use of an appropriate SBDL, developed according to the scientific criteria defined herein, could have a beneficial public health impact. Improved epidemiological links between listeriosis and the implicated food could further support this belief. The application of an SBDL for products that support rapid growth of *L. monocytogenes* at the consumer and food handler level, e.g., "use within *x* days" of opening/purchase, may have a positive impact on public health if combined with an effective educational program for temperature control at the consumer level. Research is needed to determine consumers' knowledge, attitudes, and practices in relation to SBDL (refrigeration times and temperatures) and effective formats for presenting the information to maximize the benefits of such labeling. It is necessary to demonstrate that behavioral changes can occur by application of an SBDL.

However, application of a specific SBDL (month/day/year) at the manufacturer's level is a concept that has many practical limitations. The magnitude in number, diversity, and complexity of products that exist in the market place make practical implementation on a large scale of the FSO-based SBDL difficult. Accurate information on initial levels and growth rates of *L. monocytogenes* for many formulations are lacking, and a FSO tied to a public health goal has yet to be established.

VIII. References

1. Audits International. 1999. US cold temperature evaluation. Available at: http://www.foodriskclearinghouse.umd.edu/audits_international.htm . Accessed 9 July, 2004.

2. Barbosa, W. B., L. Cabedo, H. J. Wederquist, J. N. Sofos, and G. R. Schmidt. 1994. Growth variation among species and strains of *Listeria* in culture broth. J. Food Prot. 57:765-769, 775.

3. Begot, C., I. Lebert, and A. Lebert. 1997. Variability of the response of 66 *Listeria monocytogenes* and *Listeria innocua* strains to different growth conditions. Food Microbiol. 14(5):403-412.

4. Beuchat, L.R. and R. E. Brackett 1990. Survival and growth of *Listeria monocytogenes* on lettuce as influenced by shredding, chlorine treatment, modified atmosphere packaging and temperature. J. Food Sci. 55:755-758, 870.

5. Cates, S.C., Kosa, K.M., Post, R.C., and Canavan, J. 2004. Consumer's attitudes toward open dating of USDA-regulated foods. Food Protection Trends. 24:82-88.

6. Centers for Disease Control and Prevention, Department of Health and Human Services. "Possession, Use and Transfer of Select Agents and Toxins, Interim Final Rule," 42 CFR part 1003, 67 FR 76885-76905, December 13, 2002.

7. Centers for Disease Control and Prevention, Department of Health and Human Services. "Possession, Use, and Transfer of Select Agents and Toxins, Interim Final Rule and Request for Comments," 42 CFR part 73, 68 FR 62245-62247, November 3, 2003.

8. Centers for Disease Control and Prevention. 2004. Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food – selected sites, United States, 2003. Morbid. Mortal. Wkly. Rept. 53:338-343.

9. Chen Y., K.M. Jackson, F.P. Chea, and D.W. Schaffner. 2001. Quantification and variability analysis of bacterial cross-contamination rates in common food service tasks. J. Food Prot. 64:72-80.

10. Chen, J.H. and J.H. Hotchkiss. 1993. Growth of *Listeria monocytogenes* and *Clostridium sporogenes* in cottage cheese in modified atmosphere packaging. J. Dairy Sci. 76:972-977.

11. de Jesus, A.J. and R.C. Whiting. 2003. Thermal inactivation, growth, and survival studies of *Listeria monocytogenes* strains belonging to three distinct genotypic lineages. J. Food Prot. 66(9):1611-1617.

12. Duffes, F., C. Corre, F. Leroi, X. Dousset, and P. Boyaval. 1999. Inhibition of *Listeria monocytogenes* by *in situ* produced and semipurified bacteriocins of *Carnobacterium* spp. on vacuum-packed, refrigerated cold-smoked salmon. J. Food Prot. 62:1394-1403.

13. FAO/WHO. 2004. Risk Assessment of *Listeria monocytogenes* in ready to eat foods – Technical Report. Microbiological Risk Assessment Series 5. World Health Organization. Food and Agriculture Organization of the United Nations. Geneva, Switzerland. Available at: http://www.fao.org/es/esn/food/risk_mra_listeria_report_en.stm . Accessed 20 August, 2004.

14. U.S. Food and Drug Administration. 1999. Program Information Manual, Retail Food Safety, Date Marking of Cheese. Available at: http://www.cfsan.fda.gov/~ear/ret-chdt.html. Accessed 9 July, 2004.

15. U.S. Food and Drug Administration. 2001a. Food Code, 2001. , Annex 3, page 311. U.S. Department of Health and Human Services. Public Health Service. Food and Drug Administration. Washington, D.C.

16. U.S. Food and Drug Administration. 2001b. Food Code, 2001. , Annex 3, page 309. U.S. Department of Health and Human Services. Public Health Service. Food and Drug Administration. Washington, D.C.

17. U.S. Food and Drug Administration/Food Safety and Inspection Service. 2001. Draft assessment of the relative risk to public health from foodborne *Listeria monocytogenes* among selected categories of ready-to-eat foods. Available at: http://www.foodsafety.gov/~dms/lmrisk.html . Accessed 9 July, 2004.

18. U.S. Food and Drug Administration/Food Safety and Inspection Service. 2003. Quantitative assessment of relative risk to public health from foodborne *Listeria monocytogenes* among selected categories of ready-to-eat foods. Available at: http://www.foodsafety.gov/~dms/lmr2-toc.html . Accessed 9 July, 2004.

19. Food Marketing Institute. 1995. Trends in the United States consumer attitudes and the supermarket.

20. Food Marketing Institute. 2002. Trends in the United States consumer attitudes and the supermarket.

21. Frye, C. P. 2000. Milk product pathogen sampling (unpublished data). International Dairy Foods Association, Milk Industry Foundation, National Cheese Institute, International Ice Cream Association. Washington, D.C.

22. Gill, C.O. and T. Jones. 2002. Effects of wearing knitted or rubber gloves on the transfer of *Escherichia coli* between hands and meat. J. Food Prot. 65:1045-1048

23. Gombas, D., Y. Chen, R.S. Clavero, and V.N. Scott. 2003. Survey of *Listeria monocytogenes* in ready-to-eat foods. J. Food Prot. 66:559-569.

24. Gorman, R., S. Bloomfield, and C.C. Adley. 2002. A study of cross-contamination of food-borne pathogens in the domestic kitchen in the Republic of Ireland. Int. J. Food Microbiol. 76:143-150.

25. Granum, P.E. 2001. *Bacillus cereus*, p. 373-381. *In* M.P. Doyle, L.R. Beuchat, and T.J. Montville (ed.), Food Microbiology: Fundamentals and Frontiers, 2nd Edition. ASM Press, Washington, D.C.

26. Griffith, C. and E. Redmond. 2001. Evaluating hygiene behaviour in the domestic setting and the impact of hygiene education. J. Infec. 43:70-74.

27. Hathaway, C.L. 1989. Occurrence of botulism caused by nonproteolytic strains of *Clostridium botulinum* in the United States. Memorandum to M.L. Cohen, Deputy Director, Division of Bacterial Diseases, Center for Infectious Diseases, Centers for Disease Control.

28. Health and Human Services. 1993. Biosafety in Microbiological and Biomedical Laboratories. HHS Publication No. (CDC) 93-8395. Available at: http://www.cdc.gov/od/ohs/biosfty/bmbl/bmbl3toc.htm . Accessed 9 July, 2004.

29. Humphrey, T.J. and D.M. Worthington. 1990. *Listeria* contamination of retail meat slicers. PHLS Microbiology Digest 7(3):57.

30. International Commission on Microbiological Specifications for Foods. 1996. Microorganisms in Foods 5. Characteristics of Microbiological Pathogens. Blackie Academic & Professional, London.

31. Institute of Food Technologists. 2001. Evaluation and Definition of Potentially Hazardous Foods. IFT/FDA Contract No. 223-98-2333 Task Order 4. Available at: http://www.cfsan.fda.gov/~comm/ift4-toc.html . Accessed 9 July, 2004.

32. Labuza, T. P. and L. M. Szybist. 2001. Open Dating of Foods. Food and Nutrition Press, Inc., Trumbull, CT.

33. Mead, P.S., L. Slutsker, V. Dietz, L.F. McCaig, J.S. Bresee, C. Shapiro, P.M. Griffin, and R.V. Tauxe. 1999. Food-related illness and death in the United States. Emerging Infect. Dis. 5:607-625.

34. Montville R., Y. Chen, and D. W. Schaffner. 2001. Glove barriers to bacterial cross-contamination between hands to food. J. Food Prot. 64:845-849.

35. Mossel, D.A.A. and Thomas, G. 1988. Sécurite microbiologique des plats prepares réfrigérés: recommendations en matiére d'analyse des resques, conception et surveillance du processus de preparation. [Recommendations for risk assessment, manufacturing and distribution strategies and monitoring for compliance of precooked chilled meals]. Microbiol. Aliment. Nutr. 6:289-309.

36. National Advisory Committee on Microbiological Criteria for Foods. 1990. Refrigerated Foods Containing Cooked, Uncured Meat or Poultry Products that are Packaged for Extended Refrigerated Shelf Life and That are Ready-To-Eat or Prepared with Little or No Additional Heat Treatment. Adopted January 31, 1990.

37. National Advisory Committee on Microbiological Criteria for Foods. 1991. *Listeria monocytogenes*. Int. J. Food Micro. 14:185-246.

38. National Advisory Committee on Microbiological Criteria for Foods. 1998. Hazard analysis and critical control point principles and application guidelines. J. Food Prot. 61:1246-1259.

39. National Institute of Standards and Technology. 2001. NIST Handbook 130 – 2001 Edition. Uniform Laws and Regulations in the Areas of Legal Metrology and Engine Fuel Quality. Uniform Open Dating Regulation, pp. 117-122.

40. Olsen, J. A., A . E. Yousef, and E. H. Marth. 1988. Growth and survival of *Listeria monocytogenes* during making and storage of butter. Milchwissenschaft 43:487-489.

41. Pelroy, G.A., M.E. Peterson, P.J. Holland, and M.W. Eklund. 1994. Inhibition of *Listeria monocytogenes* in cold-process (smoked) salmon by sodium lactate. J. Food Protect. 57:108-113.

42. Pinner, R.W., A. Schuchat, B. Swaminathan, P.S. Hayes, K.A. Deaver, R.E. Weaver, B. D. Plikaytis, M. Reeves, C.V. Broome, J. D. Wenger, and the *Listeria* Study Group. 1992. Role of foods in sporadic listeriosis: II. Microbiologic and epidemiologic investigation. JAMA 267:2046-2050.

43. Rosenow, E. M. and E. H. Marth. 1987. Growth of *Listeria monocytogenes* in skim, whole and chocolate milk, and in whipping cream during incubation at 4, 8, 13, 21 and 35°C. J. Food Prot. 50:452-459, 463.

44. Tompkin, R. B. 2002. Control of *Listeria monocytogenes* in the food processing environment. J. Food Prot. 65:709-725.

45. U.S. Department of Agriculture. 1997. *Pathogen Modeling Program*.USDA Agricultural Research Service, Wyndmoor, PA. Available at: http://www.arserrc.gov/mfs/pathogen.htm . Accessed 9 July, 2004.

46. U.S. Department of Agriculture/UK Foods Standards Agency/Institute of Food Research. 2003. ComBase – A combined database for predictive microbiology. Available at: http://www.combase.cc/ . Accessed 9 July, 2004.

47. Wallace, F.M., J.E. Call, A.C.S. Porto, G.J. Cocoma, The ERRC Special Projects Team, and J.B. Luchansky. 2003. Recovery rate of *Listeria monocytogenes* from

commercially prepared frankfurters during extended refrigerated storage. J. Food Prot. 66:584-591.

48. Wenrich, T., K. Cason, N. Lv, and C. Kassab. 2003. Food safety knowledge and practices of low income adults in Pennsylvania. Food Prot. Trends 23:326-335.

49. Whiting, R.C. and L.K. Bagi. 2002. Modeling the lag phase of *Listeria monocytogenes*. Int. J. Food Microbiol. 73:291-295.

Appendix I. Guidance for conducting microbial challenge studies of refrigerated RTE foods to validate an SBDL

Challenge studies have been used to determine the growth kinetics of the target microorganism(s) under conditions that mimic as closely as possible the distribution, storage and use of a food product. They may be used to establish an SBDL when mathematical microbial growth models are not available or appropriate. While studies with naturally contaminated products may be preferable, this is seldom possible with pathogens. Thus, the food must be intentionally inoculated with the target microorganism(s). For the purposes of this document, the Committee identified *L. monocytogenes* as the target organism for establishment of SBDLs. The protocol outlined here may be useful for other purposes or pathogens.

Laboratory facility and personnel. Every precaution should be taken to prevent a pathogen from contaminating a food production area. The laboratory should be constructed so that a commercial manufacturing facility cannot become contaminated with the pathogen. Challenge studies should be performed by food microbiologists who are knowledgeable in aseptic technique, the precautions necessary when handling microbial pathogens, quantitative aspects of microbiology, and fundamental microbial ecology of foods. Laboratory design and personnel protective equipment must be appropriate for the pathogen used in the study (*23*). Laboratory registration with the select agents program may be required for certain pathogens (*6, 7*).

Selection of the reference strain(s). Substantial differences among strains can occur because each can respond differently to temperature and other factors (e.g., pH, a_w) that influence its growth in a food matrix. Using at least 3 to 5 strains, individually or in combination, is recommended. The selection of strains should be based on the history and origin of the strains and on their behavior in foods. Desirable strain characteristics include the following: 1) being of clinical origin, particularly from an outbreak, 2) being of food or food environment origin, particularly if isolated from a similar food, and 3) having robust growth characteristics. Non-pathogenic surrogate strains (*e.g., L. innocua*) can be used if the strains selected have equivalent survival and growth characteristics in the food to that of the pathogenic strains.

Preparation of the inoculum. Consideration should be given to the physiological state of the contaminating cells and equivalent states should be used in the study if appropriate (e.g., acid adapted, desiccated). In many cases, the stationary phase may be selected because the contaminating cells in a production environment are more likely to be in this phase than the exponential phase. Consideration should be given to the activation of spores. If necessary, spores can be activated prior to inoculation or activated in the course of the food process.

Source of the food. The food should be as similar as possible to the product at the likely point of contamination, wherever that might be in the food chain. This includes consideration of spoilage microflora and levels likely to be present.

Inoculating and packaging the food. The level of inoculum should reflect the contamination expected to occur in the food chain. This may require enumerating the inoculum rather than the inoculated food to verify the initial level. The method of inoculation should be consistent with how the food may become contaminated. The type of food and method of packaging can influence how the food is inoculated. The method of inoculation should consider the physical state of the food, whether solid, liquid, semi-solid or multi-component. Contamination may not be homogeneous; in such cases, the inoculum need not be evenly distributed.

Consideration should be given to the temperature of the product at the point where contamination occurs. Generally the food should be pre-equilibrated to the temperature that will be used for the storage study. Cells should be in an appropriate diluent that does not influence the survival or growth in the food. The inoculum should not change the characteristics of the food or create a microenvironment. Once inoculated, the product should be packaged in the same manner as the actual product (e.g., air packed, vacuum packed, modified atmosphere packaging [MAP]). When headspace atmosphere is a factor, consideration should be given to the product surface to volume ratio within the package.

Storing the inoculated food. SBDLs are not designed to provide safety for situations of high contamination or abusive storage times; however, consideration of a range of temperatures reflecting those likely to be encountered in retailers' and consumers' refrigerators is warranted. The storage temperature should approximate the conditions that the food will normally encounter after final packaging. One approach is to use a single temperature. Another approach is to use variable temperatures for which the food will typically be exposed during the time from packaging through when the food is eaten. This is much more complex and it is questionable whether the result of the effort will yield a different public health outcome when all the factors involved in foodborne illness are considered.

Foods are exposed to a wide variety of time/temperature combinations from farm, manufacturing, distribution, display, transport, preparation, storage, and use. For most manufactured refrigerated RTE foods there is good temperature control from packaging through distribution to retail. FSIS requires that meat and poultry products be shipped at temperatures not exceeding 40°F. The 2001 Food Code has established ≤ 41°F as the appropriate temperature for storage of potentially hazardous foods. However, Audits International reported that 90% of home refrigerators in the U.S. were below 45°F (*1*). As a result, there is no universally "correct" temperature to conduct studies. There is no doubt that standardization of an approach would facilitate comparison of results. Therefore, the Committee agreed that 45°F could represent a standardized refrigeration temperature to reflect the consumer segment of refrigeration storage (e.g., for determination of a "consume within *x* days after opening").

Time interval for sampling the inoculated food. The time intervals (e.g., days, weeks) should be spaced to demonstrate: 1) whether growth occurs and 2) when the end point is reached. Typically this would involve 5 to7 time intervals. Two to three samples should be analyzed beginning on the day of inoculation and at each time interval thereafter. The information must be adequate to validate the time used for the SBDL.

Methodology for analyzing inoculated samples to measure growth. The sampling methods should be appropriate for the foods and the method of inoculation. For example, certain solid foods could be rinsed and the rinsate analyzed by direct plating onto an appropriate medium if the organism is expected to be localized to the surface. Other solid foods such as multi-component or porous foods may need to be weighed and blended with diluent. Liquid foods could be analyzed by mixing and then removing an aliquot for analysis.

Replications. It is desirable that the challenge study should be replicated a sufficient number of times with different lots or batches of product to account for product variation. This provides confidence regarding the growth kinetic values obtained. The need for replication would be reduced to the extent that data exist for similar products. Use of formulations more favorable for growth of the challenge organism would limit the need for replications of, or even testing, formulations less likely to support growth.

Documenting results of the challenge study. It is important to document the actual formula of the product tested. Also, important product characteristics (e.g., pH, water activity, proximate analysis, etc.) should be determined and recorded. The microbial test protocol used and the results of the challenge study should be documented in a report that includes data and/or a graph and the report should be retained to support the SBDL. The quantity of food to inoculate may differ but the results must be reportable on the basis of CFU/g and/or CFU/serving; otherwise it is not possible to validate the SBDL.

Appendix II. Members of the NACMCF Criteria for Refrigerated Shelf-life Based on Safety Subcommittee

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